# THE POPULATION CYTOLOGY OF *SCILLA AUTUMNALIS*

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Ph. D. Thesis

December, 1980

## ABSTRACT

Scilla autumnalis L. (the autumn squill) is a bulbous member of the Liliaceae. S. autumnalis has a circum-Mediterranean distribution and is restricted to open, free draining sites. In England S. autumnalis is a plant of open habitats in which the vegetation remains very short. Reproduction is exclusively by seed.

Cytological investigation of population samples from England, the Channel Islands, northern France, Greece and Portugal have revealed that S. autumnalis is a polyploid complex. The complex comprises six cytologically distinct races, involving two separate genomes A and B; a diploid BB (2n = 2x = 14), a diploid AA (2n = 2x = 14), an allotetraploid AABB (2n = 4x = 28), an autotetraploid BBBB (2n = 4x = 28), an autoallohexaploid AABBBB (2n = 6x = 42) and an autohexaploid BBBBBB (2n = 6x = 42). There is no homology between the two genomes and the A genome is 43% longer than the B genome. No populations have been found which contain a mixture of chromosome races.

Plants of the six chromosome races are morphologically very similar even though there has been considerable differentiation of the two genomes. A possible scheme for the evolution of the polyploid complex is proposed.

The chromosomal structure of populations of BB diploid, BBBB autotetraploid and AABBBB autoallohexaploid races has been investigated in forty-five populations from England, the Channel Islands and Corfu (Greece). The incidence of chromosomal variation, both structural and numerical is extraordinarily high in all three races.

Numerical variation comprised aneusomaty, aneuploidy, polysomaty and polyploidy. The extent of numerical variation increased with ploidy

level. Hexaploids were unstable in somatic tissues.

Structural variation in S. autumnalis is of three types; spontaneous between-cell, whole plant unique and whole plant polymorphic. The frequency of all three increases with ploidy level. Of the structural changes affecting whole plants, deletions, inversions and duplications were common but only the latter two were involved in polymorphic systems. Forty-five polymorphisms were detected, some of which were geographically widespread. Three polymorphisms were present in more than one chromosome race. The number of polymorphisms in a population is negatively correlated with latitude. Peripheral populations show less variation than central populations.

# The population cytology of *Scilla autumnalis*

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# CHAPTER ONE

## INTRODUCTION

Johannsen (1909) was the first to make the distinction between the genotype and phenotype of an individual. The genotype is the sum of the hereditary material present in an organism while the phenotype is the product of the interaction between genotype and environment. Although the phenotype changes continuously throughout the life of the individual, the genotype remains relatively constant except for rare mutations. Now individuals do not exist in isolation but are connected by breeding into loose assemblages or populations. Although the genotype of each individual in a population remains constant during its lifetime the genetic constitution of the population will change from generation to generation by the action of a number of processes: mutation, migration, random drift and natural selection.

One of the most potent forces operating in populations is natural selection. This can only be effective, however, if there is genetic variation inherent in the population. The more genetic variation present in a population, the greater is the opportunity for natural selection to act. Genetic variation occurs at the level of the gene itself and also at the level of the chromosome. Much of the variation at the chromosome level is not subject to selection directly but only indirectly via the ability to produce offspring. The distinction has been drawn here between exophenotype, affecting immediate survival, and endophenotype with effects on future generations (Lewis and John, 1963).

Information concerning the chromosomal structure of natural populations is relatively scarce. Despite the fact that the human chromosome

complement was first accurately described only in 1956 by Tjio and Levan, the population cytology of Homo sapiens is better known than that of any other organism (Hook and Porter, 1977). Such cytological studies have shown that changes in chromosome structure in humans occur with a frequency of about 1.5 per 1,000 new-born babies (Jacobs et al, 1972) and numerical changes, specifically aneuploidy, are relatively common, particularly those affecting the sex-chromosomes (Court-Brown, 1967).

Both structural and numerical chromosome variations are potent forces in evolution though the significance of structural chromosomal rearrangements in particular was dismissed as unimportant by several distinguished geneticists. Fisher in 1930 wrote "the evolutionary possibilities of these kinds of change are evidently extremely limited compared to those of the type of change to which the term gene-mutation is applied ..... only in special cases could they contribute appreciably to the genetic diversity of an interbreeding population." The importance of structural changes in evolution lies in the indirect effects on fertility and the stability and balance of genic combinations (Darlington, 1956).

Many organisms have more than two basic sets of chromosomes, a phenomenon referred to as polyploidy. Although polyploidy is uncommon in animals (White, 1973) it is very frequent in plants and has probably been involved in the origin of about a third of all species (Stebbins, 1971). Polyploidy provides an example of dramatic single step evolution referred to as saltatory evolution. The 'gigas' mutants isolated by de Vries in Oenothera were cogent evidence in favour of his mutation theory of evolution and against the Darwinian scheme. Darwinian evolution occurs by the accumulation of many small changes over a long period of time in

response to large scale environmental effects such as climatic change. Although single-gene mutations can produce dramatic change of phenotype they are generally pathological (Mather, 1953) and few convincing causes of single gene changes contributing to progressive evolution exist. Rather we envisage gradual allelic substitution, in which each novel substitution has only a small phenotypic effect, as a 'model' of Darwinian evolution.

Clearly polyploidy is thus an exception to the Darwinian view of evolution since a new species arises in a single step which may be instantly isolated from its progenitors by a sterility barrier. However, polyploidy is essentially a change in the endophenotype and although it can achieve immediate genetic isolation the genetic balance and hence external phenotype may be little altered (Lewis and John, 1963). Indeed, gigas effects are usually absent in natural polyploids. In certain cases two species, or races of a single species, may hybridise and chromosome doubling will lead to balanced individuals carrying both genomes. Many such hybridisations followed by chromosome doubling may take place involving several diploids and many ploidy levels. In this way a polyploid complex is built up (Babcock and Stebbins, 1938).

Although the evolutionary history of a number of polyploid complexes has been documented, such as Scilla scilloides (Haga and Noda, 1976), Tragopogon (Ownbey, 1950) and the wheat group (Riley, 1965) few investigations of the population structure of the individuals comprising a complex have been attempted.

Scilla autumnalis (Liliaceae) is a widespread and locally common species with a circum-Mediterranean distribution. The species is composed of a number of cytologically-distinct races and forms a polyploid complex.

It is hoped that the detailed study of populations of the races of Scilla autumnalis presented here will provide an insight into both the chromosomal structure of natural populations and the evolution of the complex itself.

# CHAPTER TWO

## THE PLANT

### Introduction

There has been much confusion over the generic delimitation of Scilla and indeed natural hybrids occur between S. bifolia and Chionodoxa luciliae (x Chionoscilla allenii) calling into doubt the biological validity of this generic distinction. However about 100 species have been described (Clapham, Tutin and Warburg, 1962) distributed throughout Europe, Africa and temperate Asia. All Scilla species are bulbous perennials producing a raceme or corymb of solitary flowers with free perianth parts which are usually blue or purple. Many species are cultivated for ornament notably S. sibirica Andr.

The species of the Mediterranean region have recently been classified from karyological and morphological evidence into a number of species groups (Greilhuber and Speta, 1976). Basic chromosome numbers in these species range from n = 4 to n = 11 (Table 2.1). The Scilla autumnalis group, subgen. Prospero (Salisb.) Chouard, contains two species: Scilla autumnalis L. (x = 7) and Scilla obtusifolia Poiret (x = 4) which are characterised by flowering in the late summer or autumn before the leaves appear, the flowers bractless and roots perennial (McNeill, 1979).

Scilla autumnalis L. (the Autumn Squill) is a bulbous perennial with bulbs up to 4.5 cm. in diameter (Plate 2.1 a). In north-western Europe flowering stems are produced in July or August and flowering continues into early September in Britain. The onset of flowering is later in more southerly populations and in Greece, for example, flowering does not begin until October. A single bulb can produce up to six flower

Table 2.1 The basic chromosome numbers of the species groups in the genus Scilla (Greilhuber and Speta, 1976)

| Chromosome basic number (x) | Species group |
|---|---|
| 4 | *Scilla persica* HAUSSKN. |
| 5 | *S. hohenackeri* FISCH. & MEY. |
| 5 | *Puschkinia* ADAMS |
| 6 | *S. siberica* HAW. in ANDR. |
| 4,7 | *S. autumnalis* L. |
| 8 | *S. peruviana* L. |
| 8 | *Endymion* DUMORT |
| 9 | *S. scilloides* DRUCE |
| 9 | *S. bifolia* L. (Incl. *Chionodoxa* BOISS.) |
| 10 | *S. hyacinthoides* L. |
| 10 | *S. verna* HUDS. |
| 10 | *Urginea* STEINH. |
| 11 | *A. atropatana* GROSSH. |

Plate 2.1 The morphology of Scilla autumnalis

(a) Scilla autumnalis

(b) Variation in flower colour

Plate 2.1 continued

(c) Plant with four inflorescences

(d) Plant with ripe capsules showing 5 or 6 seeds in a capsule

spikes in one season though the average is 2 - 3 (Plate 2.1c). Individual spikes range from 4 to 25 cm. in length at anthesis. The inflorescence is a simple raceme with 4 - 40 flowers (Plate 2.1b, Fig. 2.1). The length of time between opening of the first and last flowers in the raceme varies between 5 and 14 days depending on the time of year and numbers of flowers in the inflorescence. The anthers dehisce a few hours after flower-opening and are borne on 3 - 5 mm. filaments (Fig. 2.1). The six free perianth parts of the flower (4 - 7.5 mm.) are lanceolate and acute with a prominent midrib. The flowers are borne on ascending pedicels 2 - 5 mm. in length (Fig. 2.3). The subgenus Prospero is supposedly characterised by the absence of bracts. In this study, however, small bracts of 1 - 5 mm. in length have been observed in plants of three cytological races.

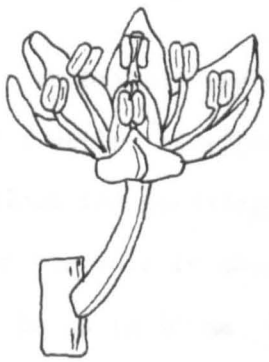
Flower colour is variable: pink to lilac perianth parts with a deeper midrib and purple anthers or white perianth parts and yellow anthers (Plate 2.1b). A single style emerges from the centre of the white or blue trilocular ovary (Fig. 2.1). The fruit is an ellipsoid capsule about 2 mm. in diameter with three loculi each containing a maximum of two seeds (Plate 2.1d).

The leaves are linear (4 - 20 cm. x 1 - 5 mm.) and are produced after the flowers. In populations towards the south of the range, the leaves begin to die down in April. In British populations, however, the leaves may persist all the year round, although a new group is produced after flowering.

Life history or phenology of the taxon

Reproduction of S. autumnalis is exclusively by seed. Only two cases of bulb division were found in about 1500 wild-collected plants. This rare bulb division both in the wild and in cultivation apparently always follows

Fig. 2.1 The morphology of Scilla autumnalis

Flower x 2

Pistil x 3

Stamen x 3

damage to the bulb by invertebrates.

S. autumnalis is predominantly an outbreeder through most plants will set some seed on self-fertilisation (Chapter 7). Pollination of S. autumnalis is by insects, mainly Hymenopterans (bumble bees, honey bees) and Dipterans although the flowers are also visited by a variety of Lepidopterans. Syrphids are the most common visitors to the flowers in England though these are probably not important pollinating agents. Examination of syrphids trapped after visits to Scilla flowers has shown little pollen adhering to legs or body even though many hundreds of grains may have been eaten. In contrast, other Dipterans eat fewer pollen grains and carry more on their legs. Insects are probably attracted to Scilla flowers by colour as there is no detectable nectar or scent.

Up to six seeds are set per flower though the average is less than this (Chapter 7). A single plant can produce up to 1000 seeds in one flowering season (Plate 2.1d) under greenhouse conditions but in nature there is considerable variation in both the number of flowers produced and the numbers of seeds set.

In Britain the seeds germinate in the spring as there is a requirement for a period of after-ripening and/or chilling. Under greenhouse conditions the seedling develops the first true leaf after about two months, when the bulb is about 5 mm. in diameter. After six months the bulb may be up to 10 mm. in diameter with five leaves. In cultivation, flowering may occur when the plant is two years old although in the wild the time from seedling establishment to flowering is probably much longer.

## Distribution

### a) World distribution

In the south of the range the species occurs in North Africa and its southern limit corresponds roughly with the 20°C January isotherm (Fig. 2.2). The most northerly populations are those in southern England and this limit corresponds with the 10°C January isotherm. It is not known how far eastwards the species extends from the Caucasus region of Russia or the Middle Eastern countries bordering the Mediterranean.

### b) British distribution

In Britain S. autumnalis is confined to a small number of suitable sites in southern England (Fig. 2.3). With the exception of single populations at Hampton Court, Surrey, Orsett, Essex, St. Helen's, Isle of Wight and the Avon Gorge, Bristol, the localities are confined to the coasts of Devon and Cornwall.

## The Habitat

Scilla autumnalis in England is a plant of open habitats in which the vegetation remains very short, generally less than six inches in height, as a result of climatic or ecological factors other than grazing. In England south of a line between the Rivers Thames and Severn, suitable habitats are provided by coastal areas in the West Country and a few areas of river gravel in the Thames basin and the Isle of Wight where the free draining nature of the sites ensure that the vegetation remains short (Plate 2.2 a-c). The coastal sites are similarly restricted to free draining exposed cliff tops. There is no strict correlation of this species with geology although the physical properties of some rock types allow a more frequent development of suitable edaphic conditions (Table 2.2). Interestingly, populations of S. autumnalis are

Fig. 2.2 The distribution of Scilla autumnalis. 10° and 20° mean January isotherms are shown.

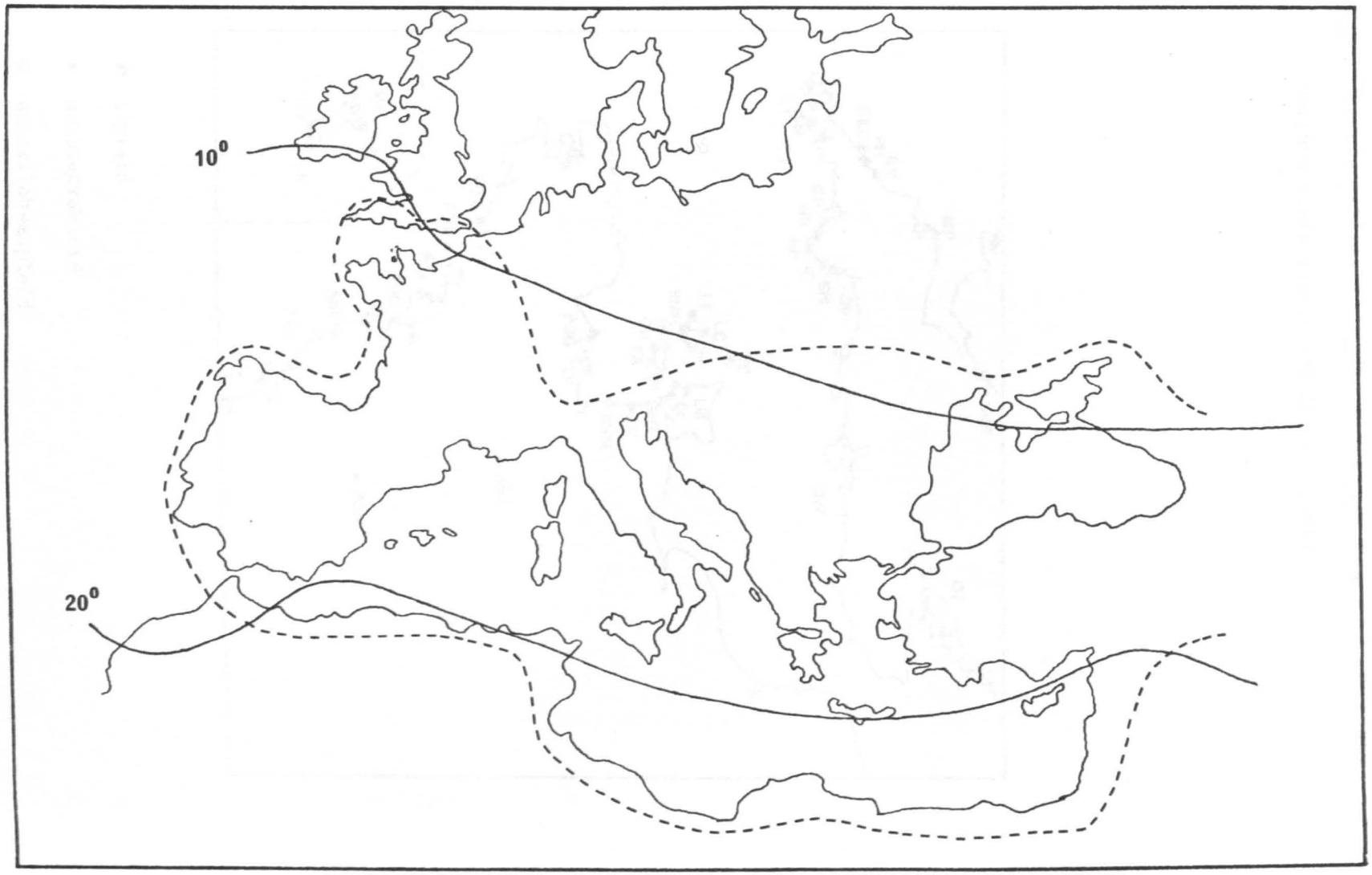
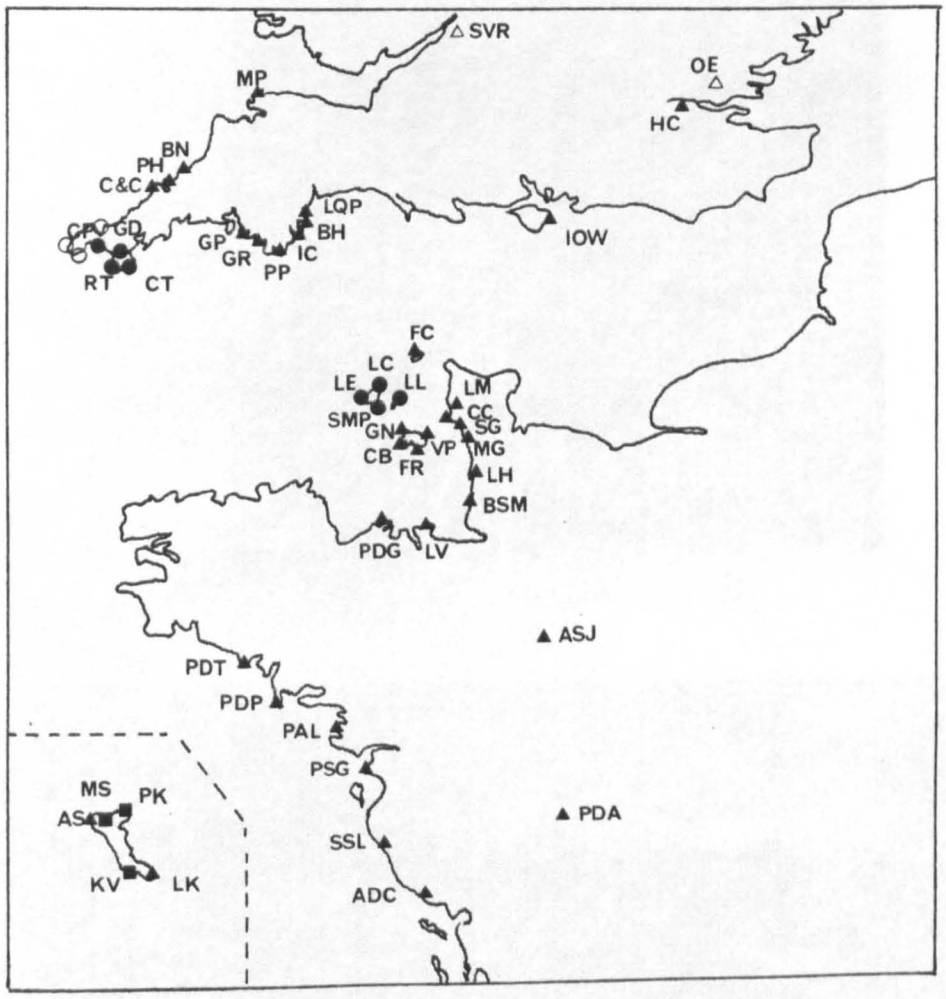


Fig. 2.3 The populations of Scilla autumnalis sampled in England, north-western France and Corfu (inset)




■ diploid

▲ autotetraploid

● autoallohexaploid

Open symbols represent populations which have not been sampled.

OE = Orsett, Essex; SVR = St. Vincent's Rocks, Bristol.

Plate 2.2 Habitats of Scilla autumnalis

(a) Pentire Head - a coastal site

(b) St. Helen's (Isle of Wight) - a site on stabilised sand

Plate 2.2 continued

(c) Hampton Court - a river gravel site

Table 2.2 Summary of population samples and habitat descriptions

| Population | Code | Latitude Longitude | Rock type | Habitat | Aspect | Estimated area ($m^2$) | Population size (estimated) | No. Plants collected |
|---|---|---|---|---|---|---|---|---|
| Diploid populations | | | | | | | | |
| Meseena, Corfu | MS | 39° 45'N 19° 42' 23"E | Breccia of black limestone | Olive grove | S | 200 | small | 27 |
| Mt. Pantokrator, Corfu | PK | 39° 45'N 19° 52' 9"E | Limestone | Rocky mountain-side | N | 10,000 | medium | 30 |
| Lake Korillion, Corfu | LK | 39° 26' 48"N 19° 55' 15"E | Alluvial silt | Field | W | 2,000 | medium | 24 |
| Tetraploid populations | | | | | | | | |
| England | | | | | | | | |
| Morte Point, Ilfracombe | MP | 51° 11' 15"N 4° 10'W | Morte slates | Cliff turf | SW | 500 | medium | 28 |
| Barras Nose, Tintagel | BN | 50° 40' 12"N 5° 45' 20W | Devonian slate | " " | " | 2,500 | large | 32 |
| Pentire Head, Polzeath | PH | 50° 35' 17"N 4° 56'W | Basaltic lava | " " | " | 1,150 | " | 30 |
| Cow and Calf, Trenance | C+C | 50° 29' 24"N 5° 2'W | Devonian slate | " " | S | 150 | small | 34 |
| Gara Point, Newton Ferrers | GP | 50° 17' 24"N 4° 4' 3"W | " " | " " | " | 750 | large | 29 |
| Gunrow's Down, Newton Ferrers | GR | 50° 17' 18"N 4° 1' 48"W | " " | " " | " | 250 | small | 32 |
| Prawle Point, Kingsbridge | PP | 50° 12' 6"N 3° 43' 24"W | Hornblende Schist | " " | " | 1,000 | large | 32 |
| Ivy Cove, Dartmouth | IC | 50° 20' 54"N 3° 30' 57"W | Devonian slate | " " | SE | 100 | small | 33 |
| Berry Head, Brixham | BH | 50° 23' 52"N 3° 29' 10"W | Devonian limestone | " " | " | 3,500 | large | 53 |
| Long Quarry Point, Torquay | LQP | 50° 28' 36"N 3° 30' 13"W | " " | " " | " | 2,500 | large | 32 |
| St. Helens, Isle of Wight | IOW | 50° 41' 48"N 1° 6' 11"W | Stabilised sand | Short grass | - | 4,000 | large | 36 |
| Hampton Court, Kingston | HC | 51° 23' 24"N 0° 19' 24"W | River gravel | " " | - | 150 | small | 30 |

/continued

Table 2.2 continued

| Population | Code | Latitude Longitude | Rock type | Habitat | Aspect | Estimated area ($m^2$) | Population size (estimated) | No. plants collected |
|---|---|---|---|---|---|---|---|---|
| Channel Islands | | | | | | | | |
| Fort Corbelets, Alderney | FC | 49° 12' 12"N 2° 10'W | Granite diorite | Cliff turf | N | 200 | small | 30 |
| Grosnez Point, Jersey | GN | 49° 15' 37"N 2° 14' 45"W | Cadomien granite | " " | NW | 1,000 | large | 30 |
| Corbière, Jersey | CB | 49° 10' 58"N 2° 14' 30"W | " " | " " | S/SW | 1,500 | medium | 31 |
| Fort Regent, Jersey | FR | 49° 10' 49"N 2° 6' 33"W | Dioritic gneiss | Short grass | W | 50 | small | 30 |
| Verclut Point, Jersey | VP | 49° 13' 31"N 2° 0' 59"W | Cadomien granite | Cliff turf | S | 500 | " | 31 |
| France | | | | | | | | |
| Les Miêlles, Surtainville | LM | 49° 27' 5"N 1° 49' 32"W | Stabilised sand | Short grass | W/SW | 200 | small | 7 |
| Cap de Carteret, Carteret | CC | 49° 22' 13"N 1° 47' 56"W | Feldspathic sandstone | Cliff turf | SW | 200 | " | 30 |
| St. Georges de la Rivière, Barneville | SG | 49° 20' 49"N 1° 44' 35"W | Stabilised sand | Sand dunes | SW | 10,000 | large | 151 |
| Mon Griffon, Portbail | MG | 49° 20'N 1° 42' 41"W | " " | " " | SW | 1,000 | medium | 30 |
| Les Houghes, Coutainville | LH | 49° 5' 17"N 1° 36' 18"W | " " | Fixed dunes | W | 5,000 | large | 27 |
| Bréville-sur-mer, Granville | BSM | 48° 52'N 1° 34' 36"W | " " | " " | N/NW | 10,000 | " | 31 |
| Le Verger, Cancale | LV | 48° 52' 10"N 1° 53' 2"W | Micaschist | Short grass | N | 5,000 | small | 29 |
| Plage de Guen, Erquy | PDG | 48° 38' 32"N 2° 29' 1"W | Feldspathic sandstone and conglomerate | " " | W | 5,000 | " | 26 |
| Pointe de Talud, L'Orient | PDT | 47° 42' 30"N 3° 26'W | Alk.granite with biotite and muscovite | Long grass and gorse | SW | 10,000 | medium | 30 |
| Pointe du Percho, Quiberon | PDP | 47° 31' 37"N 3° 9' 26"W | " " | Fixed dunes | SW | 40,000 | large | 30 |
| Port au Loup, Piriac-sur-mer | PAL | 47° 23' 24"N 2° 31' 36"W | Micaschist | Long grass | NW | 1,500 | small | 22 |
| Pointe de St. Gildas, Pornic | PSG | 47° 8' 8"N 2° 14' 34"W | Feldspathic gneiss | Long grass and gorse | S | 2,000 | " | 24 |
| Sion-sur l'oceon, St. Gilles sur Vie | SSL | 46° 42' 36"N 1° 58' 40"W | Sericitic and chloritic schists | Cliff turf | W/SW | 50 | " | 31 |

Table 2.2 continued

| Population | Code | Latitude Longitude | Rock type | Habitat | Aspect | Estimated area ($m^2$) | Population size (estimated) | No. plants collected |
|---|---|---|---|---|---|---|---|---|
| Anse de Cayola, les Sables d'Olonne | ADC | 46° 26' 26"N 1° 42' 52"W | Micaschist | Cliff turf | SW | 100 | small | 28 |
| Pont de l'Argenton, Massais | PDA | 47° 0' 16"N 0° 20' 14"W | Gabbro | Grassy stope | SW | 600 | " | 36 |
| Argentré-sur-Jouanne, Laval | ASJ | 48° 3' 36"N 1° 7' 23"W | Laval schist | Rocky stope | SW | 750 | medium | 32 |
| Corfu | | | | | | | | |
| Aghios Stephanos | AS | 39° 45'N 19° 38' 1"E | Breccia of limestone | Cliff turf | S | 2,000 | small | 23 |
| Kavos | KV | 39° 22' 5" 20° 8' 17"E | " " | Olive grove | E | 1,000 | large | 23 |
| Hexaploid populations | | | | | | | | |
| England | | | | | | | | |
| Cudden Point, Marazion | CP | 50° 6' 14"N 5° 9' 18"W | Greenstone | Cliff turf | S/SE | 300 | medium | 34 |
| Caerleon Cove, The Lizard | CT | 49° 59' 45"N 5° 10'W | Serpentenite | " " | SE | 300 | " | 30 |
| Goonhilly Downs, The Lizard | GD | 50° 2' 50"N 5° 9' 18"W | " | Grass and gorse | - | 150 | " | 31 |
| Rill Ledges, The Lizard | RT | 49° 58' 18"N 5° 13' 10"W | " | Cliff turf | SW | 300 | " | 35 |
| Channel Islands | | | | | | | | |
| Lancresse Common, Guernsey | LC | 49° 29' 30"W 2° 30'W | Granite diorite | Short grass | NW | 50,000 | large | 28 |
| L'Ereé, Guernsey | LE | 49° 27'N 2° 39' 18"W | Gneiss and Amphibolites | Long grass | W | 250 | medium | 28 |
| St. Martin's Point, Guernsey | SMP | 49° 25'N 2° 30' 42"W | " " | Cliff turf | E | 1,000 | large | 31 |
| Les l'Aches, Sark | LL | 49° 25'N 2° 19' 30"W | " " | Cliff turf | S/SE | 800 | medium | 29 |

not found on areas of chalk even where the habitat requirements are apparently fulfilled.

All populations in coastal areas of Devon and Cornwall are found on slopes where the aspect has a southerly component even in populations on the north coasts of Devon and Cornwall (S, SW or SE; Table 2.2, e.g. Plate 2.2a). This requirement for a south-facing slope is presumably linked to temperature, with north-facing slopes being too cold for survival or reproduction of S. autumnalis.

As one moves south in the range of S. autumnalis the habitat preferences become less marked and inland sites are more common. Coastal populations in France are less restricted to sites in cliff turf and are also found on fixed dunes and amongst denser vegetation, even growing with shrubs such as Ulex and Calluna. Site aspect is no longer exclusively south, south east or south west. All the sites studied, including those in Corfu, had free draining soils. The only geological pre-requisite for Scilla sites is, then, that the rock weathers to produce such a soil.

In English coastal sites Scilla autumnalis is commonly found associated with Plantago lanceolata and Festuca rubra (Table 2.3). Other frequently associated species are Leontodon hispidus, Hypochoeris radicata, Dactylis glomerata, Lotus corniculatus, Agrostis stolonifera, Plantago coronopus and Anthyllis vulneraria.

Population sampling

Bulbs of S. autumnalis were collected at random from each population. The number of bulbs collected varied with population size but was usually about 30 (Table 2.2). The minimum distance between bulbs sampled was 3 m. and, where possible, 5 m. This was dependent on the size and density of individuals in the population.

Table 2.3 The occurrence of associated plant species with Scilla autumnalis in three tetraploid and three hexaploid populations, recorded as frequency in 25 x 25 cm. quadrats.

| Species | Tetraploid | | | Hexaploid | | |
|---|---|---|---|---|---|---|
| | BN | BH | PH | CT | GD | RT |
| *Plantago lanceolata* | 6 | 9 | 1 | 6 | 4 | 7 |
| *Festuca rubra* | 15 | 12 | 5 | 1 | - | 5 |
| *Leontodon hispidus* | 14 | 3 | 1 | 1 | - | 1 |
| *Hypochoeris radicata* | 1 | 1 | 4 | 1 | 4 | - |
| *Dactylis glomerata* | 3 | 4 | 1 | 1 | - | 1 |
| *Lotus corniculatus* | 3 | 1 | - | - | 1 | 1 |
| *Agrostis stolonifera* | 1 | 1 | - | 1 | - | 1 |
| *Plantago coronopus* | 8 | 1 | 9 | - | - | 9 |
| *Anthyllis vulneraria* | 2 | 1 | 2 | 6 | - | - |
| *Koeleria cristata* | - | 10 | - | 4 | - | 8 |
| *Daucus carota* | 6 | - | - | 3 | - | 1 |
| *Galium saxatile* | - | - | - | 4 | 1 | 3 |
| *Sedum anglicum* | 8 | - | 9 | 1 | - | - |
| *Thymus drucei* | - | 8 | - | - | 1 | 1 |
| *Armeria maritima* | 4 | - | - | 3 | - | 1 |
| *Plantago maritima* | 1 | - | - | - | 3 | 10 |
| *Taraxacum officinale* | - | 6 | - | - | 1 | - |
| *Agrostis setacea* | - | - | - | - | 10 | 2 |
| *Aira caryophyllea* | 8 | - | - | - | 1 | - |
| *Trifolium repens* | 1 | 3 | - | - | - | - |
| *Bellis perennis* | - | 5 | - | - | - | 2 |
| *Carex flacca* | - | 5 | - | - | - | 1 |
| *Ulex europaeus* | - | - | - | - | 2 | 1 |
| *Cerastium holosteoides* | - | 1 | - | 1 | - | - |
| *Trifolium sp.* | - | - | - | 1 | - | 2 |
| *Calluna vulgaris* | 1 | - | - | - | - | 1 |
| *Jasione montana* | - | - | 2 | - | - | - |
| *Bromus sp.* | - | - | 2 | - | - | - |
| *Betonica officinalis* | - | - | 1 | - | - | - |

/continued

Table 2.3 continued

| Species | Tetraploid | | | Hexaploid | | |
|---|---|---|---|---|---|---|
| | BN | BH | PH | CT | GD | RT |
| *Polygonum aviculare* | - | 1 | - | - | - | - |
| *Poterium sanguisorba* | - | 12 | - | - | - | - |
| *Galium aparine* | - | 3 | - | - | - | - |
| *Aira praecox* | - | - | - | - | - | - |
| *Hieracium pilosella* | - | 3 | - | - | - | - |
| *Achillea millefolium* | - | 2 | - | - | - | - |
| *Carex sp.* | - | 4 | - | - | - | - |
| *Medicago lupulina* | - | 2 | - | - | - | - |
| *Spiranthes spiralis* | - | 1 | - | - | - | - |
| *Centauria scabiosa* | - | 1 | - | - | - | - |
| *Sonchus oleraceus* | - | 3 | - | - | - | - |
| *Holcus lanatus* | 1 | - | - | - | - | - |
| *Chrysanthemum leucanthemum* | - | - | - | 2 | - | - |
| *Allium schoenoprasum* | - | - | - | - | 4 | - |
| *Molinia caerulea* | - | - | - | - | 1 | - |
| *Silene maritima* | 1 | - | - | - | - | - |
| *Anthemis arvensis* | - | - | - | - | - | 2 |
| *Centaurium sp.* | - | - | - | - | - | 1 |
| Total quadrats | 39 | 39 | 23 | 26 | 44 | 30 |
| Total number of associated species | 19 | 26 | 11 | 15 | 12 | 21 |

Most populations sampled are estimated to have in excess of 1,000 individuals and many are extremely large with upwards of 100,000 individuals.

A total of 46 populations have been studied: 16 in southern England, 16 in north-western France (Brittany and Normandy), 9 in the Channel Islands and 5 on the island of Corfu, Greece (Fig. 2.3, Table 2.2).

# CHAPTER 3

## THE CHROMOSOMES OF SCILLA AUTUMNALIS

### A. The chromosome races

The specific epithet Scilla autumnalis refers to a polyploid complex with diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) levels of ploidy. The complex has been the subject of some cytological investigations (Battaglia, 1952, 1957, 1959, 1963, 1964; Gimenez-Martin, 1959; Heitz, 1926; Maude, 1939, 1940; McNeill and Knight, 1962; Mordak, 1971; Neves, 1974; Ruiz Rejón et al., 1980; Tarnavschy, 1948), though many authors have merely recorded a chromosome count (Table 3.1).

Six chromosome races have been identified, involving two distinct genomes, and two of these races are reported in this thesis for the first time. A diploid race BB (Fig. 3.2b) is found towards the southern limit of the distribution in North Africa, southern Spain, Sardinia, Sicily, Greece and Israel. An autotetraploid race BBBB (Fig. 3.2d) extends from the Mediterranean up through Europe to southern England, the northern limit of the distribution of the species (Fig. 3.1; Table 3.1). Diploid and autotetraploid races overlap in Sardinia and Corfu and possibly in southern Spain, southern Italy and mainland Greece. In Corfu the two races are separated by as little as 10 km.

A single population of a second diploid race AA (Fig. 3.2a) has been found on the coast of Portugal. This population is closely surrounded by an allotetraploid race AABB (Fig. 3.2c) which is the only other race found so far in Portugal. The full extent of the allotetraploid distribution is as yet unknown.

Two distinct hexaploids have been detected: an autohexaploid BBBBBB (Fig. 3.2f) and an autoallohexaploid AABBBB (Fig. 3.2e). The autohexaploid

Table 3.1 Localities of plants of Scilla autumnalis whose chromosomes have been counted.

| Race | Country | Locality | Author |
|---|---|---|---|
| AA | Portugal | Peniche, Leiria | This thesis |
| BB | Algeria | Bone, Bon Zizi | Battaglia (1952) |
| | Greece | Corfu (3 localities, Table 2.2) | This thesis |
| | Israel | Jerusalem | Battaglia (1957) |
| | | Tel Aviv | " " |
| | Italy | Pantellaria | " (1964) |
| | | Sicily (Catania and Scicli) | " (1957) |
| | | Sardinia (Bosa) | " (1964) |
| | Malta | | " " |
| | Morocco | Ain Sebaa | " (1957) |
| | Spain | Albeaquemada | Gimenez-Martin (1959) |
| | | Serra de Cazorla, Jaén | Ruiz Rejón (1980) |
| | Tunisia | Tunis | Battaglia (1957) |
| | Turkey | Istambul | " " |
| BBBB | France | Cahors | Battaglia (1957) |
| | | Cannet des Mahres, Var | " " |
| | | Creux de Meje, Montpellier | Kosta (1972) |
| | | Corsica (Bonifacio) | Battaglia (1964) |
| | | Normandy (6 localities, Table 2.2) | This thesis |
| | | Brittany (4 localities, Table 2.2) | " " |
| | | Maine (1 locality, Table 2.2) | " " |
| | | Poitou (5 localities, Table 2.2) | " " |
| | Gt.Britain | S. England (14 localities, Table 2.2) | " " |
| | | Channel Islands (Jersey, Alderney) | " " |
| | Greece | Corfu (2 localities, Table 2.2) | " " |
| | Italy | Scardaville, Emilia | Battaglia (1957) |
| | | Arbule, Lazio | " " |
| | | S. Guiseppe Cairo, Liguria | " " |
| | | Balza Rossa, Marche | " " |
| | | Martina Franca, Puglia | " " |
| | | Arneo, Puglia | " " |
| | | Dossi da Vollunga, Trentino | " " |
| | | San Daniele, Trieste | " " |
| | | Venizia Guilia, Trieste | " " |
| | | S. Guiliano, Tuscany | " " |
| | | S. Rossare, Tuscany | " " |
| | | Verruca, Tuscany | " " |
| | | Sardinia (Cagliari) | Martinoli (1949) |
| | | " (Cuglieri) | Battaglia (1964) |
| | | " (Laconi) | " " |
| | | " (Sas Aroleddas) | " " |
| | | " (Tresnuraghes) | " " |

/cont...

Table 3.1 continued

| Race | Country | Locality | Author |
|---|---|---|---|
| BBBB | Spain | Perdon | This thesis |
| | | Majorca (Sta.Margherita) | Battaglia (1964) |
| | | Ibiza (Balneario) | " " |
| | Yugoslavia | Kolar, Montenegro | Natarajan (1974) |
| | | Ulcinj, " | " " |
| AABB | Portugal | Portinho do Arrabida, Setubal | This thesis |
| | | Villa Nogueira de Arrabida, Setubal | " " |
| | | Serra de Montejunto, Lisbon | " " |
| | | Reguengo Grande, Leiria | " " |
| | | Serra dos Candieros, Santarem | " " |
| | | Foz do Arelho, Leiria | " " |
| | | Minde, Santarem | " " |
| | | Fatima, Leiria | " " |
| | | Condeixa, Coimbra | " " |
| | | Cabo Mondego, Coimbra | " " |
| BBBBBB | Hungary | Lake Balaton | Baksay (1956) |
| | Italy | Carso | Battaglia (1964) |
| | | Malfalcone, Doberdo | " " |
| | | Monte S. Daniele, Trieste | " " |
| AABBBB | Gt.Britain | England (S.W.Cornwall; 7 locations; Table 2.2) | This thesis |
| | | Channel Islands (Guernsey and Sark; Table 2.2) | " " |

Fig. 3.1 The distribution of the chromosome races of Scilla autumnalis

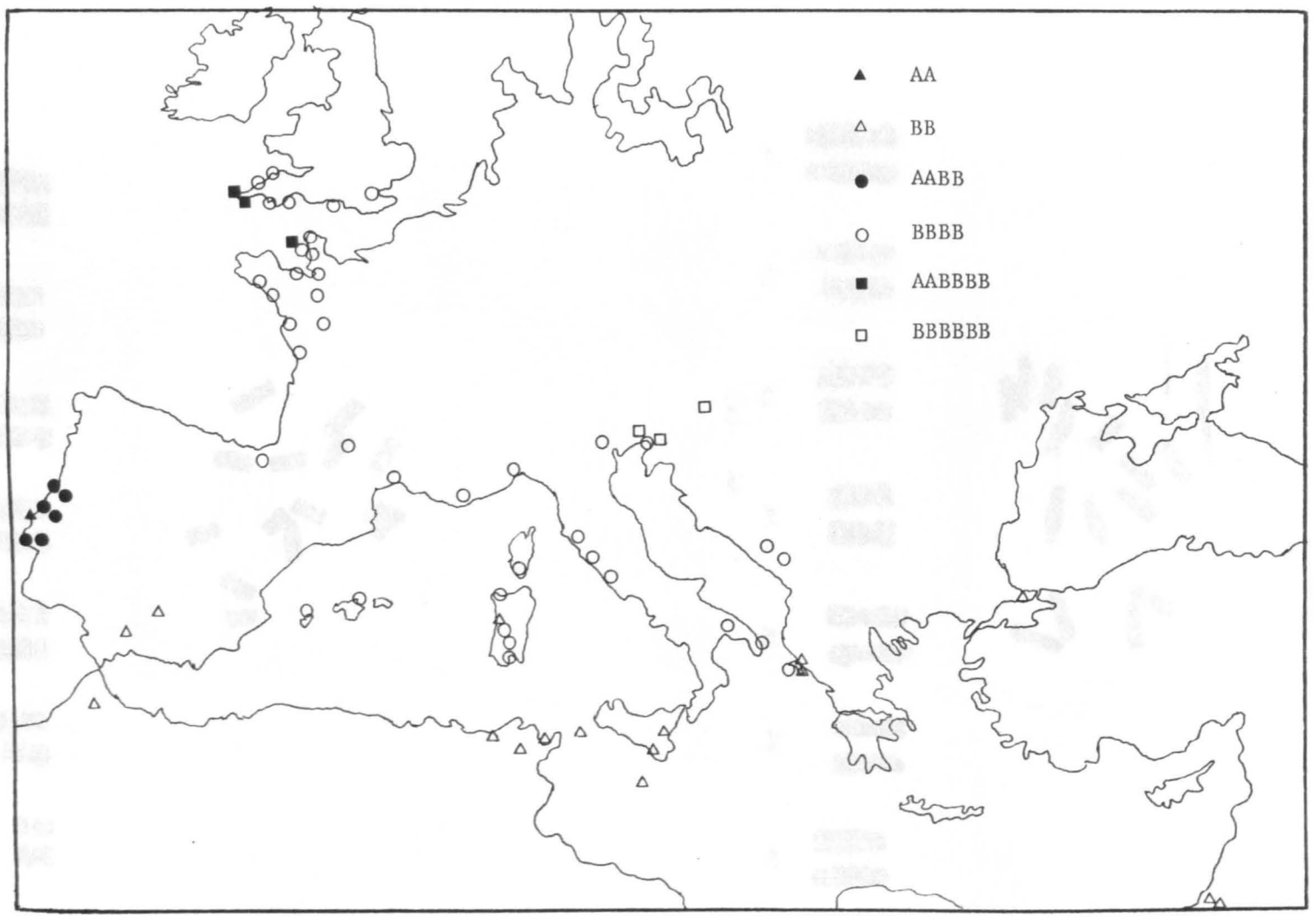
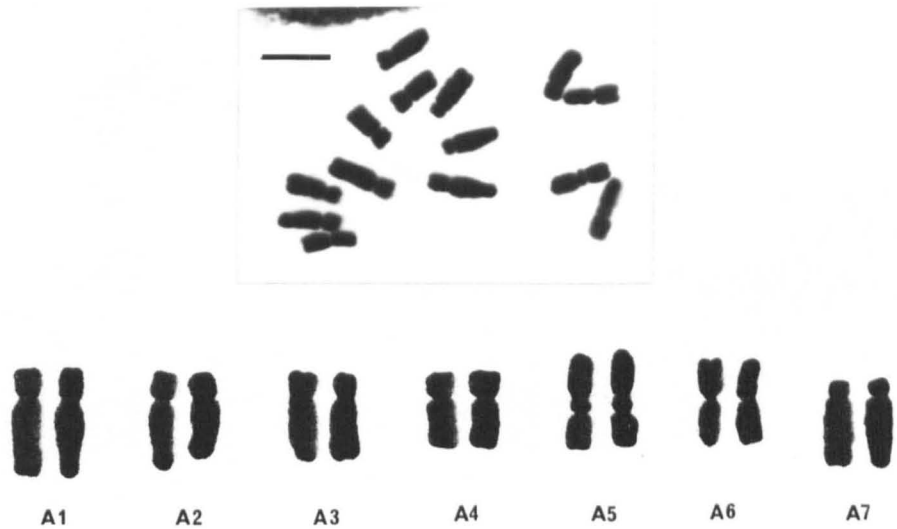
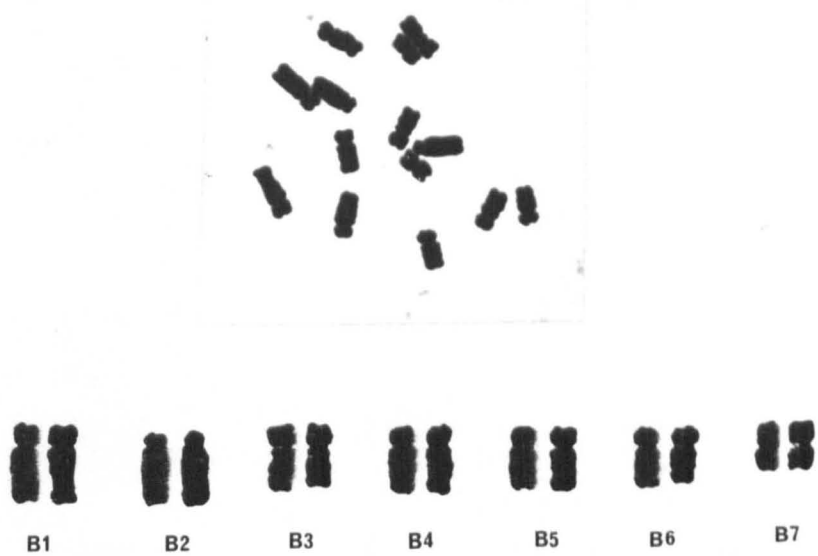


Figure 3.2 The karyotypes of the chromosome races of Scilla autumnalis

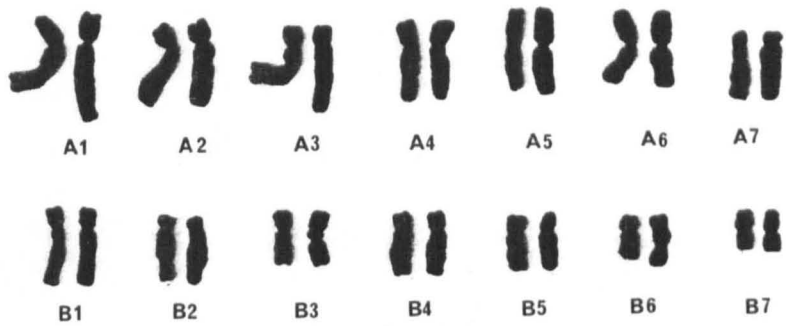



(a) AA

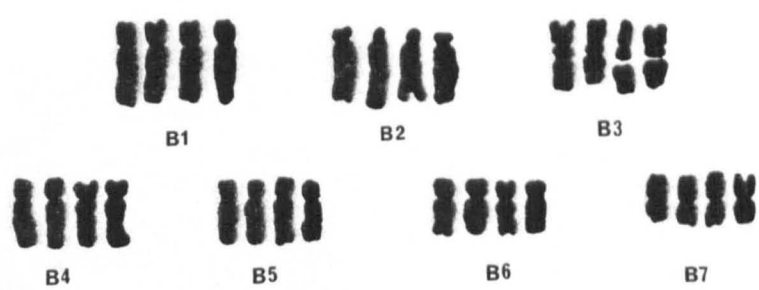



(b) BB

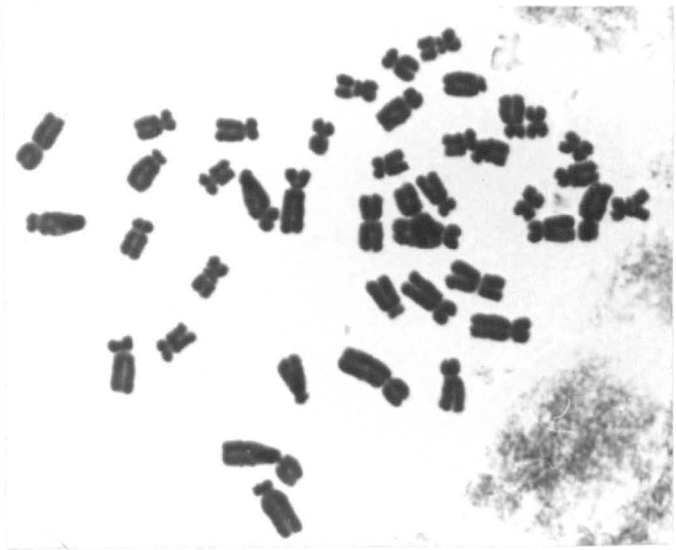
Bar in all photographs represents 10 μm

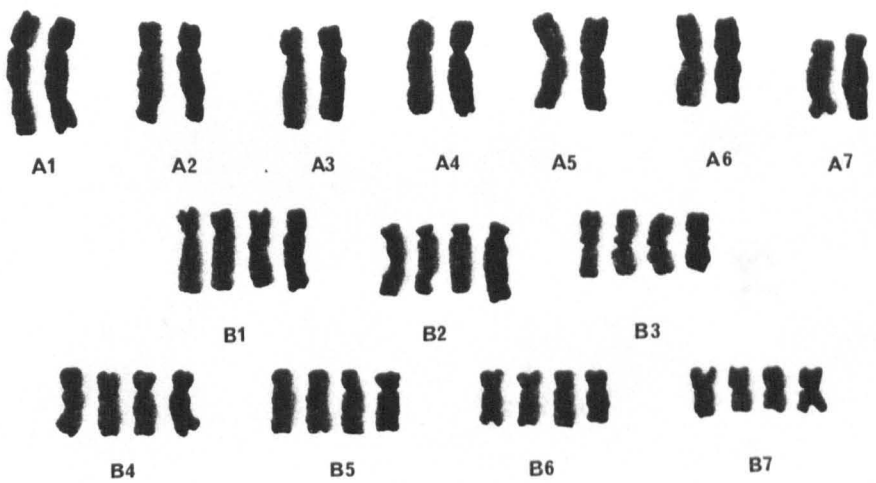



(c) AABB

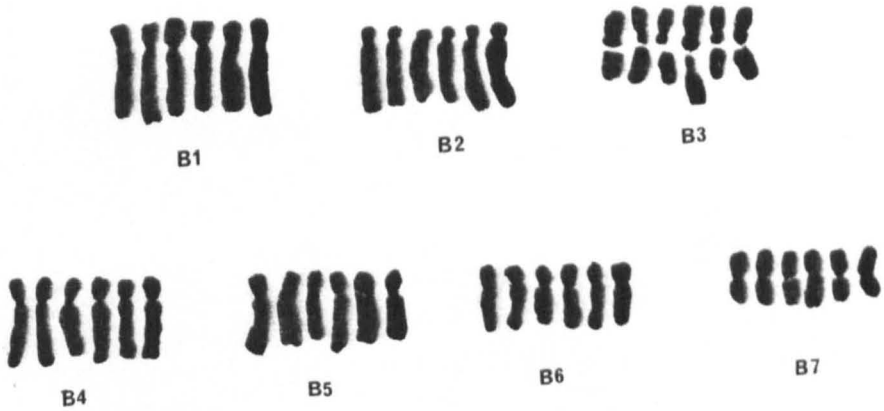



(d) BBBB






(e) AABBBB




(f) BBBBBB

occurs in northern Italy near Trieste and in Hungary and may be widespread in the Balkans. The autoallohexaploid, by contrast, is confined to Guernsey and Sark in the Channel Islands and the south-western peninsula of Cornwall (the West Penwith and Lizard Peninsulas).

In this thesis populations of BB diploids and BBBB autotetraploids from Corfu, autotetraploids from England and north-western France and autoallohexaploids from Cornwall and the Channel Islands have been investigated (Fig. 2.3).

B. The chromosome complements

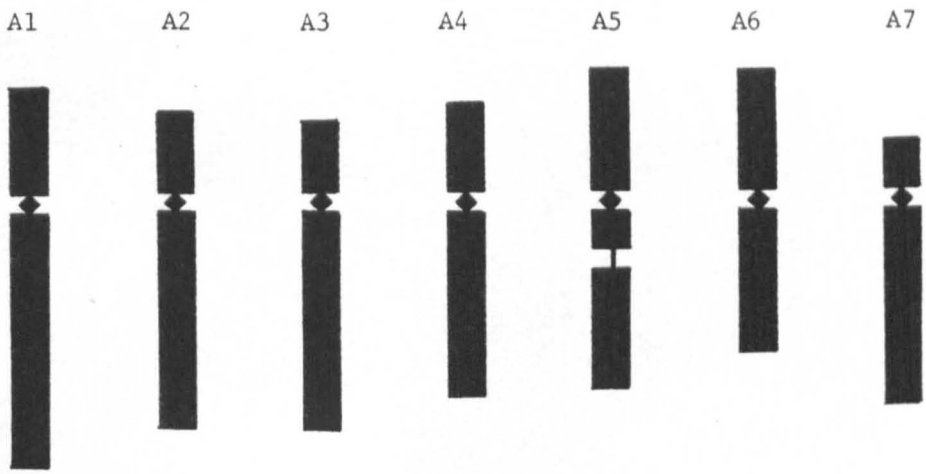
I. The A genome

The chromosomes of Scilla autumnalis designated as the A genome were unknown until this thesis. The chromosomes range from 8.02 to 12.3 μm in length at colchicine metaphase (Fig. 3.3) and the total haploid mitotic length is 67.05 μm.

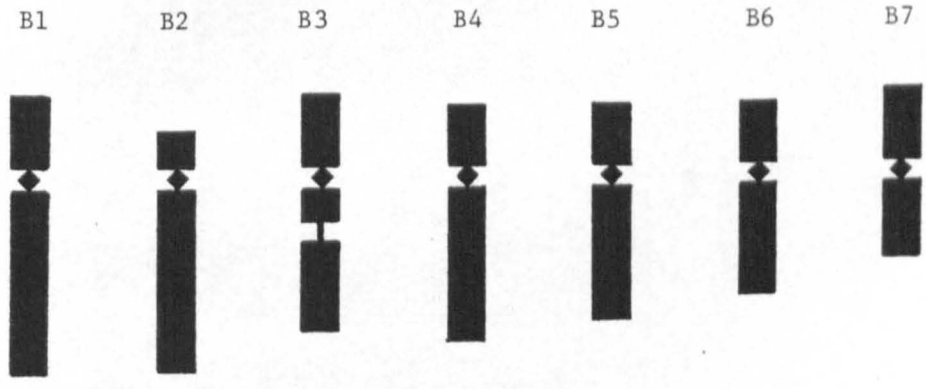
Chromosomes A1 - A4 and A7 are acrocentric while A5 and A6 are nearly metacentric. Chromosome A1 is the largest in the complement (12.3 μm) with an arm ratio of 1:2.29. Pairs A2 and A3 are indistinguishable acrocentrics about 10 μm in length with arm ratios of 1:2.6. Chromosome A4 is shorter (9.1 μm) and more submetacentric with an arm ratio of 1:2.03. Chromosomes A5 and A6, both nearly metacentric, are about 9.00 μm in length with arm ratios of about 1:1.2. Chromosome A5 is the nucleolar-organiser chromosome, the N.O.R. being located in the longer arm about one quarter of the length distant from the centromere. Finally, chromosome A7 (8.02 μm) is highly acrocentric having the largest arm ratio in the complement (1:4.26).

Chromosome A5, the nucleolar-organiser chromosome, is particularly conspicuous by virtue of the adjacent primary and secondary constrictions.

Fig. 3.3 Karyotypes of the A and B genomes of Scilla autumnalis at colchicine metaphase (mean values of 20 cells).




| | A1 | A2 | A3 | A4 | A5 | A6 | A7 |
|---|---|---|---|---|---|---|---|
| Short arm (μm) | 3.74 | 2.76 | 2.51 | 3.00 | 4.03 | 4.03 | 1.53 |
| Long arm (μm) | 8.56 | 7.28 | 7.18 | 6.10 | 5.17 | 4.67 | 6.49 |
| Total (μm) | 12.30 | 10.04 | 9.69 | 9.10 | 9.20 | 8.70 | 8.02 |
| Arm ratio | 1:2.29 | 1:2.64 | 1:2.86 | 1:2.03 | 1:1.28 | 1:1.16 | 1:4.26 |




| | B1 | B2 | B3 | B4 | B5 | B6 | B7 |
|---|---|---|---|---|---|---|---|
| Short arm (μm) | 2.41 | 1.18 | 2.36 | 2.02 | 2.02 | 2.02 | 2.46 |
| Long arm (μm) | 6.15 | 6.05 | 4.08 | 5.17 | 4.53 | 3.74 | 2.46 |
| Total (μm) | 8.56 | 7.23 | 6.44 | 7.19 | 6.55 | 5.76 | 4.92 |
| Arm ratio | 1:2.55 | 1:5.13 | 1:1.73 | 1:2.56 | 1:2.24 | 1:1.90 | 1:1.00 |

In combinations with the B genome such as AABB and AABBBB, however, the N.O. region is suppressed and chromosomes A5 and A6 cannot then be reliably distinguished. Nucleolar-organiser suppression will be discussed in Chapter 6.

II. The B genome

The B genome chromosomes (Fig. 3.3) are smaller than the A genome chromosomes with the single exception of B1 (8.56 µm) which is slightly longer than chromosome A7 (8.02 µm). The total haploid mitotic length of the B genome is 46.73 µm, 30.3% shorter than the A genome.

The seven chromosomes of the haploid set are all distinguishable during mitotic metaphase in good preparations. Chromosomes B1 - B6 are acrocentric while B7 is metacentric. Chromosome B1, the largest chromosome (8.56 µm), has an arm ratio of 1:2.55. Chromosome B2 (7.23 µm) is highly acrocentric with an arm ratio of 1:5.13. Chromosome B3, the nucleolar-organiser chromosome, has an arm ratio of 1:1.73. The nucleolar-organiser region is located in the long arm about one-third of the length distant from the centromere. In common with the A genome N.O. chromosome, this chromosome is highly conspicuous in mitotic preparations. Chromomeres are sometimes visible within the secondary constriction.

Chromosomes B4, B5 and B6 are 7.18, 6.54 and 5.76 µm in length with arm ratios of 1:2.56, 1:2.24 and 1:1.90 respectively. Interestingly, the short arm of each is 2.02 µm in length. Chromosome B7, the smallest in the complement (4.92 µm), is metacentric.

Chromosome width is significantly smaller in the B genome than in the A genome in cells of the AABBBB race ($F = 6.91$, $P < 0.001$; Tables 3.2 and 3.3). Variation in chromosome width between cells in the same study is also significant reflecting differences in contraction ($F = 23.36$, $P < 0.001$; Tables 3.2 and 3.3).

Table 3.2 Average widths of A and B genome chromosomes in 9 cells of autoallohexaploids. (Fourteen A and fourteen B genome chromosomes measured in each cell)

| Genome | Chromosome | Mean width (μm) | s.d. | Mean width per genome (μm) |
|---|---|---|---|---|
| A | 1 | 2.58 | 0.5 | |
| | 2 | 2.51 | 0.42 | |
| | 3 | 2.53 | 0.35 | |
| | 4 | 2.60 | 0.43 | 2.55 |
| | 5 | 2.57 | 0.46 | |
| | 6 | 2.51 | 0.37 | |
| | 7 | 2.56 | 0.43 | |
| B | 1 | 2.41 | 0.48 | |
| | 2 | 2.48 | 0.38 | |
| | 3 | 2.41 | 0.40 | |
| | 4 | 2.47 | 0.41 | 2.39 |
| | 5 | 2.38 | 0.38 | |
| | 6 | 2.34 | 0.37 | |
| | 7 | 2.30 | 0.35 | |

Table 3.3 Analysis of variance of chromosome width in the A and B genomes of autoallohexaploids. (Fourteen A and fourteen B genome chromosomes measured in each cell)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between cells | 8 | 34.91 | 4.36 | 23.36 | <0.001 |
| Between genomes within cells | 9 | 1.68 | 0.187 | 6.91 | <0.001 |
| Between chromosomes within genomes | 234 | 6.33 | 0.027 | | |
| Total | 251 | 42.92 | | | |

C-banding techniques have revealed no detectable heterochromatin in A or B genomes. In addition, the only chromocentres detectable in interphase nuclei are very small, few in number and nucleolus-associated.

C. Meiosis

The early stages of meiotic prophase are tangled and not suitable for definitive study but paired threaois can be followed at pachytene (Plate 3.1a). Chiasma number can be determined at late diplotene/early diakinesis in diploid plants (Plate 6.4a) but this is not possible in tetraploids. All studies of the distribution and frequency of chiasmata have been made on metaphase-I PMCs when centromere co-orientation allows recognition of long and short arms (Plate 3.1 b-g).

i) Diploids (BB)

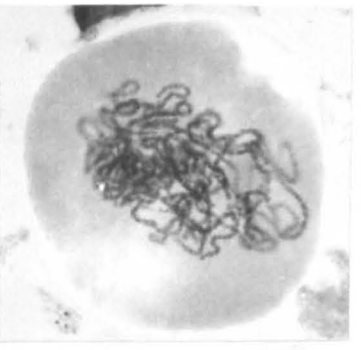
At metaphase-I four bivalent classes are distinguishable (Plate 3.1c). Acrocentric chromosome 1 forms distinctly the largest bivalent and metacentric chromosome 7 the smallest. Chromosome 2 is highly acrocentric with a very small, rarely chiasmate, short arm. Acrocentric chromosomes 3-6 form a group of similar sized unidentifiable bivalents.

Two plants with standard complements were scored for chiasma frequency and distribution. Mean chiasma frequencies in LK3 and MS18 were 15.32 and 14.0 respectively (Table 3.4). The chiasma numbers per cell were variable and ranged from 11-17 and 11-19 respectively. Univalents have rarely been observed in diploids.

Chiasma frequency in the four bivalent groups is closely related to chromosome length (Table 3.4). The regression of chiasma frequency per bivalent on mitotic chromosome length is significant and positive ($r = 0.807$, $P < 0.001$; Fig. 3.4).

In bivalents 1-6 chiasmata can be allotted to long and short arms. Interestingly, the short arm of bivalent 2 is chiasmate less frequently

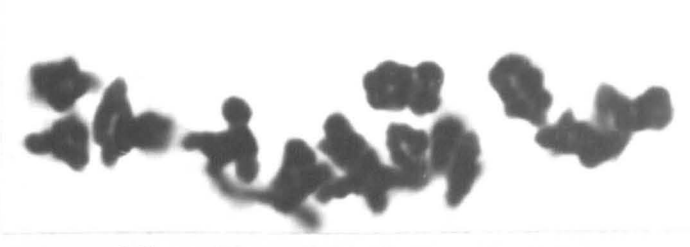
Plate 3.1 Meiosis in the chromosome races of Scilla autumnalis



(a) Pachytene in BBBB

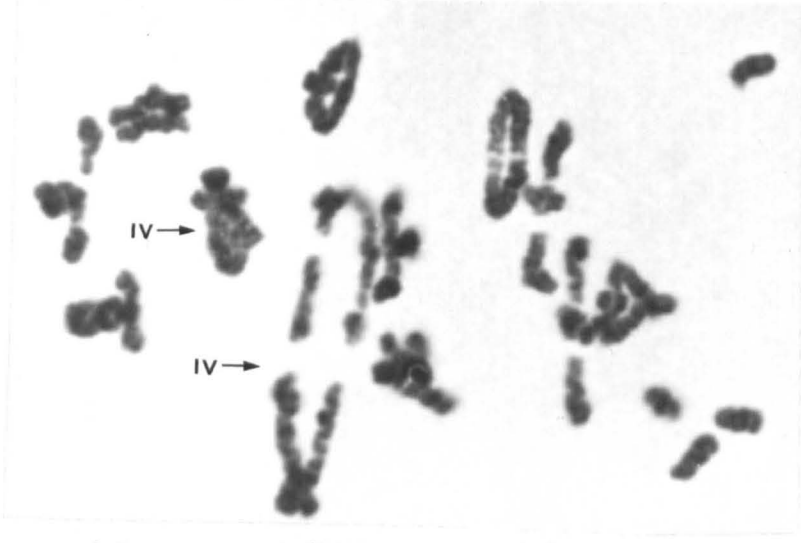
(b) AA M-I (7II + 1B)

(c) BB M-I (7II)

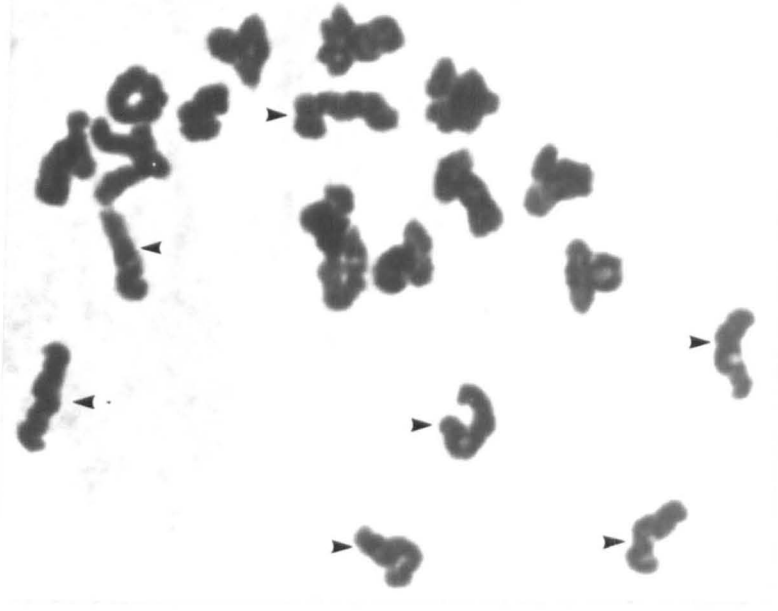


(d) AABB M-I (14II)

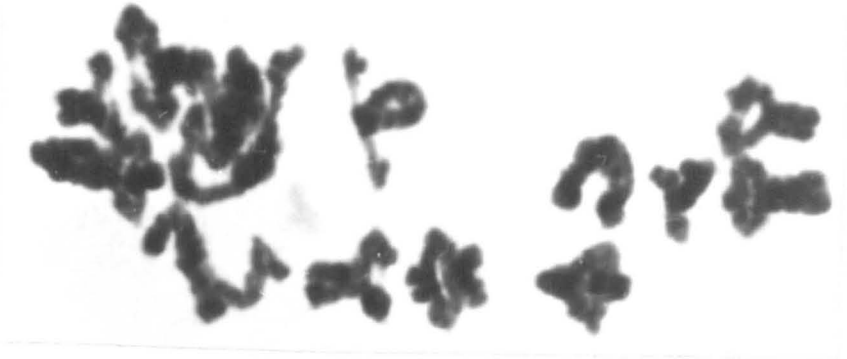
Plate 3.1 continued




(e) BBBB M-I (10II + 2IV + 4B)



(f) $F_1$ hybrid ABBBB (14B II + 7AI)



(g) AABBBB M-I

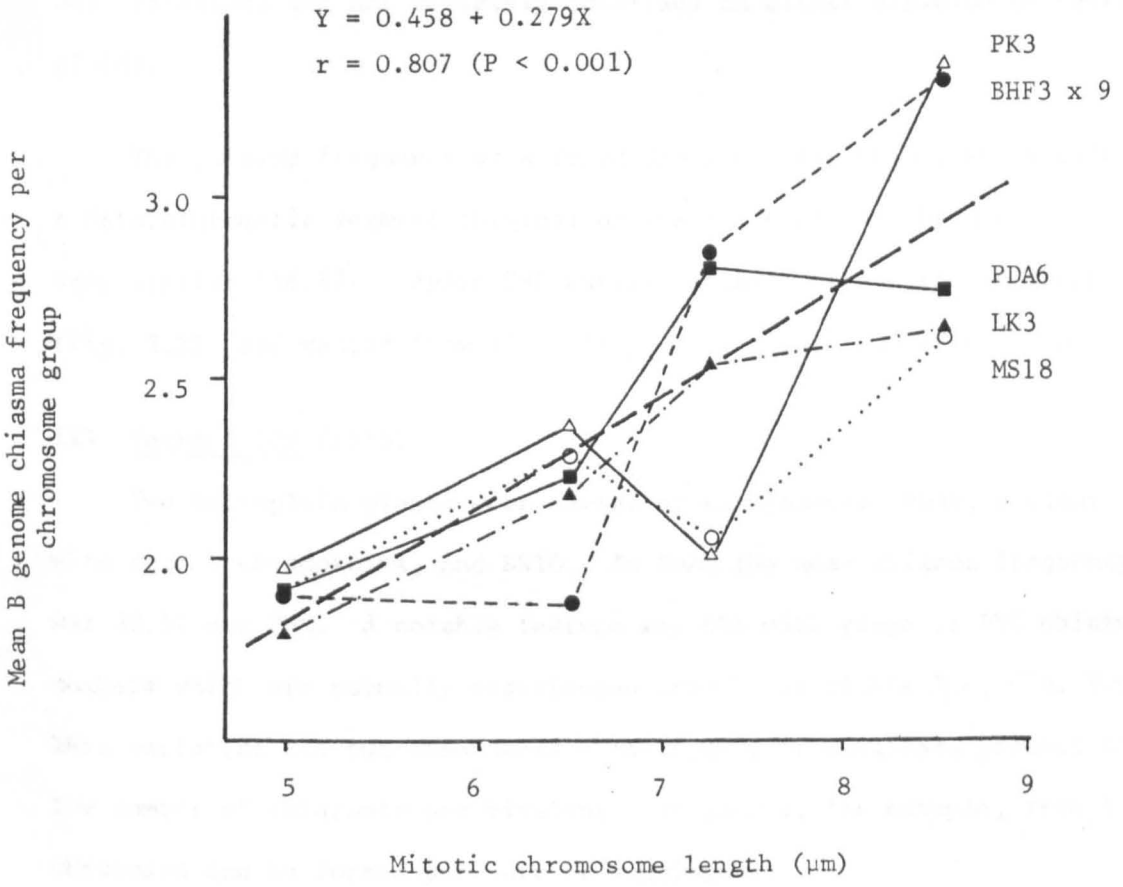
Table 3.4 Chiasma frequency of diploid, autotetraploid, $F_1$ hybrid and autoallohexaploid plants of S. autumnalis

| | | Chiasmata per PMC | | | | Mean B genome chiasma frequency per chromosome group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant | Ploidy level | Mean | Range | S.D. | No. PMCs | B1 | B2 | B3-6 | B7 | Total |
| LK3 | 2x | 15.32 | 11-17 | 1.89 | 19 | 2.63 | 2.53 | 8.76 | 1.79 | 15.32 |
| MS18 | 2x | 14.0 | 11-19 | 1.87 | 21 | 2.62 | 2.05 | 7.48 | 1.91 | 14.0 |
| PK3* | 2x | 16.87 | 11-21 | 1.87 | 53 | 3.34 | 2.00 | 9.52 | 1.98 | 16.87 |
| PDA6 | 4x | 32.54 | 27-39 | 2.90 | 50 | 2.72 | 2.80 | 8.92 | 1.92 | 16.24 |
| BN10 | 4x | 25.0 | 21-28 | 2.32 | 11 | - | - | - | - | 12.5 |
| BHF3 x 9** | 5x | 34.2 | 26-44 | 4.80 | 20 | 3.30 | 2.83 | 9.12 | 1.93 | 17.10 |
| LL11 | 6x | 42.0 | 25-54 | 10.99 | 25 | - | - | - | - | 12.5 |

* Duplication heterozygote

** ABBBB hybrid

Fig. 3.4 Regression of mean B genome chiasma frequency per chromosome group on mitotic chromosome length for five plants of different ploidy level (data from Table 3.3).



than the short arm of bivalent 1 and the difference is greater than expected on the basis of length and bivalent chiasma frequency. Thus, although the chiasma frequency of each bivalent is dependent on overall mitotic length, the incidence of short arm chiasmata is not length-dependent and other factors, such as the mode of pairing initiation, must play a role in the allocation of chiasmata within the genome.

Although detailed analysis of chiasma position has not been carried out, chiasmata are not obviously localised in either diploids or tetraploids.

The chiasma frequency of a third diploid plant (PK3), which carries a heterochromatic segment terminal on the short arm of chromosome 2, was very similar (16.87). Again, PMC chiasma numbers were normally distributed (Fig. 3.5) and ranged from 11 to 21 per cell (see Chapter 6, p. 203).

ii) Tetraploids (BBBB)

Two tetraploid plants were scored at metaphase-I: PDA6, a plant with four B-chromosomes, and BN10. In PDA6 the mean chiasma frequency was 32.54 per PMC. A notable feature was the wide range of PMC chiasma numbers which are normally distributed from 27-39 (Table 3.4, Fig. 3.6). This variation has two components - the number of bivalents present and the number of chiasmata per bivalent. In pair 1, for example, from 1 to 5 chiasmata can be formed per pair of homologues.

BN10 has a lower chiasma frequency than PDA6 (Table 3.4) with a mean of 25.0.

Mitotic studies indicate that the 28-chromosome race is autotetraploid and quadrivalent formation at meiosis provides additional evidence for this contention (Tables 3.5 and 3.6). All seven chromosomes of the haploid set

Fig. 3.5 Frequency distribution of PMCs with different numbers of chiasmata in a diploid plant (PK3)

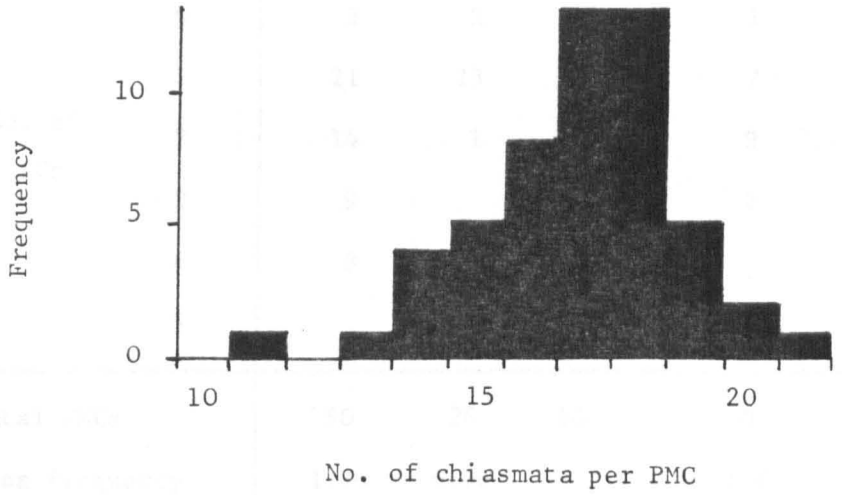



Fig. 3.6 Frequency distribution of PMCs with different numbers of chiasmata in the autotetraploid PDA6

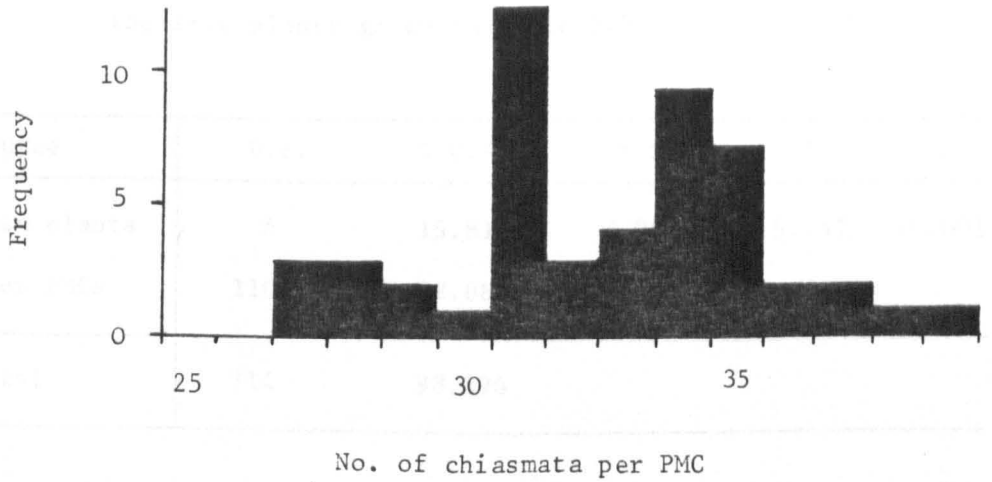


Table 3.5 Frequency of B genome quadrivalents in five plants of S. autumnalis

| | | Numbers of PMCs | | | | |
|---|---|---|---|---|---|---|
| | Plant | PDA6 | BN10 | MP18 | BHF3 x 9 | LL11 |
| No. of IVs | 0 | 3 | 2 | 1 | 1 | 2 |
| | 1 | 21 | 23 | 6 | 7 | 5 |
| | 2 | 14 | 1 | 3 | 9 | 2 |
| | 3 | 9 | | | 2 | |
| | 4 | 3 | | | | |
| | 5 | | | | 1 | |
| Total PMCs | | 50 | 26 | 10 | 20 | 9 |
| Mean frequency | | 1.76 | 0.96 | 1.2 | 1.8 | 1.0 |
| S.D. | | 1.021 | 0.344 | 0.632 | 1.056 | 0.707 |

Table 3.6 Analysis of variance of B genome quadrivalent frequency of the five plants given in Table 3.5

| Source | D.F. | S.O.S. | M.S. | F | D |
|---|---|---|---|---|---|
| Between plants | 4 | 15.814 | 3.954 | 5.247 | <0.001 |
| Between PMCs | 110 | 82.882 | 0.754 | | |
| Total | 114 | 98.696 | | | |

Bartlett's test for homogeneity of variance $\chi^2_{(4)} = 9.23$ ($P > 0.05$)

are involved in quadrivalent formation. Quadrivalents in groups 1, 2 and 7 can be unequivocally identified, and a single cell was observed with four quadrivalents, all of bivalent group 3-6. The maximum number of quadrivalents observed in a single PMC was four.(Plate 3.1e).

Quadrivalent frequency is clearly dependent on chiasma frequency and was higher for PDA6 (1.76) than for BN10 (0.96). Thus, on average, each cell in tetraploid plants has at least one quadrivalent. As in diploids univalents were rare, only 0.04 per cell in PDA6 (Table 3.7) and none observed in BN10. No trivalents, therefore, or associations of more than four were observed in tetraploids (Table 3.7).

Quadrivalents are not equally distributed amongst the seven chromosome groups ($\chi^2_{(3)} = 11.89$, $P < 0.01$; Table 3.8). This deviation can be attributed to chromosome groups 2 and 7. Group 2, despite a high chiasma frequency, forms few quadrivalents. The short arms of this homologous group rarely form chiasmata and this will limit the probability of quadrivalent formation. Group 7 chromosomes, the smallest in the complement, have the lowest chiasma frequency (Table 3.4), and are seldom involved in associations of four. In tetraploids as in diploids, chiasma frequency is positively correlated with mitotic length ($r = 0.807$, $P < 0.001$, Fig. 3.4).

iii) Hexaploids (AABBBB) and Pentaploids (ABBBB)

Meiotic analysis has been carried out on one autoallohexaploid from Sark (LL11) and one synthetic pentaploid hybrid with a genomic constitution ABBBB. In neither plant was pairing between A and B genomes observed.

a) Hexaploids

Plant LL11 from Sark had an overall mean chiasma frequency of 42.0 (Table 3.9). This can be partitioned into 25.0 and 17.0 for the tetraploid

Table 3.7 Metaphase-I pairing patterns in diploid, tetraploid, pentaploid and hexaploid plants

| Ploidy level | Plant | Chromosome association | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| 2 x (BB) | PK3 | 0.038 | 6.981 | | | |
| | LK3 | 0.095 | 6.952 | | | |
| | MS18 | - | 7.00 | | | |
| 4 x (BBBB) | PDA6 | 0.04 | 10.38 | - | 1.80 | - |
| | BN10 | - | 12.00 | - | 1.00 | - |
| 5 x (BBBBB) | PSG26 | 2.444 | 7.444 | 3.444 | 0.444 | 1.111 |
| (ABBBB) | BHF3 x 9 | - | 10.50 | - | 1.75 | - |
| 6 x (AABBBB) | LL11 | 0.889 | 10.667 | - | 1.444 | - |

Table 3.8 The numbers of quadrivalents involving the different chromosome groups in autotetraploid PDA6 and $F_1$ hybrid BHF3 x 9 (ABBBB). The expected values are calculated from an assumption of equal frequency per group.

| Plant | | Quadrivalents per chromosome group B1 | B2 | B3-6 | B7 | Total quadrivalents |
|---|---|---|---|---|---|---|
| PDA6 | observed | 15 | 6 | 64 | 5 | 90 |
| | expected | 12.86 | 12.96 | 51.43 | 12.86 | 90 |
| | | $\chi^2_{(3)} = 11.89$ ($P < 0.01$) | | | | |
| BHF3 x 9 | observed | 5 | 0 | 27 | 3 | 35 |
| | expected | 5 | 5 | 20 | 5 | 35 |
| | | $\chi^2_{(3)} = 8.25$ ($P < 0.05$) | | | | |

Table 3.9 Chiasma frequency in an autoallohexaploid plant (LL11)

| Genome | Frequency Mean | Range | s.d. | Mean chiasma frequency per homologous pair | No. PMCs |
|---|---|---|---|---|---|
| AA | 17.0 | 11-24 | 4.472 | 2.43 | 9 |
| BBBB | 25.0 | 13-33 | 7.228 | 1.79 | 9 |
| Total | 42.0 | 25-54 | 10.989 | - | 9 |

BBBB and diploid AA components respectively. The average chiasma frequency of the A bivalents then is 2.43, 35% greater than that of homologous pairs of the B genome (1.79). A maximum of six chiasmata were observed in a single A bivalent compared with 4 in a B bivalent. Individual bivalents were not identified in this study. Chiasma number per PMC was very variable for both the AA and BBBB components and combined chiasma numbers ranged from 25-54 per cell.

In contrast to diploids and autotetraploids, univalents are relatively common in hexaploids, the mean number per PMC being 2.44 in LL4 (Table 3.7).

Between 0 and 2 B genome quadrivalents were found per cell with a mean of 1.0 (Table 3.5). No chiasmate association between A and B chromosomes either as heteromorphic bivalents or associations of more than four were observed thus emphasising the autoallohexaploid nature of the plant (Table 3.7; Plate 3.1g).

b) Pentaploids

Pentaploids (2n = 5x = 35) of constitution ABBBB were synthesised by crossing autotetraploids (BBBB) with autoallohexaploids (AABBBB).

In the pentaploids seven A chromosomes were present as univalents in all PMCs while the four B genomes behaved regularly (Plate 3.1f). Mean chiasma frequency was 34.2 (Table 3.4), only slightly (and non-significantly) higher than that of the tetraploid plant PDA6 (see above). Chiasma numbers ranged from 26-44 per PMC. The distribution of chiasmata between the four identifiable chromosome groups of genome B again showed the same positive length relationship as diploids and autotetraploids (Fig. 3.4; Table 3.4).

The number of quadrivalents per cell varied between 0 and 5 with a mean of 1.8 (Table 3.5) similar to the tetraploid and hexaploid plants.

No B genome univalents were observed in a large sample of PMCs. The distribution of quadrivalents between the chromosome groups is again non-random ($\chi^2_{(2)} = 8.25$; $P < 0.05$) due largely to a deficiency in group 2 (Table 3.8). The effects of the A univalents in ABBBB hybrids on the later stages of meiosis and the implications of their behaviour will be discussed later (Chapter 7).

Chiasma frequency in the hybrid plant BHF3 x 9 was not significantly different from the tetraploid PDA6 (Table 3.4). In addition, B genome pairing patterns in all plants with four B genomes (autotetraploids, autoallohexaploids and $F_1$ hybrids) were similar (Table 3.7). This is particularly interesting since in the $F_1$ hybrid the B genomes originate from two distinct ploidy levels yet behave in an identical fashion to B genomes in autotetraploids and autoallohexaploids. This will be discussed further in Chapter 7.

iv) AA diploids and AABB allotetraploids

In AA diploids chiasma frequency is about 50% higher than in BB diploids with a mean of about 3 per bivalent (Parker, pers. comm; Plate 3.1b).

In allotetraploids pairing is strictly homologous with 14 bivalents in all PMCs (Plate 3.1d). Again, the A bivalents have higher chiasma frequencies than the B bivalents.

B genome chiasma frequency and ploidy level

Comparison of the meiotic behaviour of B genomes at different ploidy levels shows significant differences in both mean chiasma frequency ($F = 15.76$, $P < 0.001$; Table 3.10) and mean quadrivalent frequency ($F = 5.247$, $P < 0.001$; Table 3.6) between plants. Multiple range analysis

Table 3.10 Analysis of variance and multiple range analysis of chiasma frequency per BB genome between seven plants of different ploidy level (data from Table 3.4)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between plants | 6 | 300.850 | 50.142 | 15.764 | <0.001 |
| Between cells | 175 | 556.646 | 3.181 | | |
| Total | 181 | 557.496 | | | |

Bartlett's test for homogeneity of variance $\chi^2_{(6)} = 2.738$ ($P > 0.8$)

Multiple range analysis

| BN10 | LL11 | MS18 | LK3 | PDA6 | PK3 | BHF3 x 9 |
|---|---|---|---|---|---|---|
| (4x) | (6x) | (2x) | (2x) | (4x) | (2x) | (5x) |

(Table 3.10) demonstrates that these differences are not related to the level of ploidy and presumably reflect variation in the genotypic component of pairing control.

# CHAPTER FOUR

## NUMERICAL VARIATION IN SCILLA AUTUMNALIS

### Introduction

Wilson (1896) wrote, "Every species of plant or animal has a fixed and characteristic number of chromosomes, which regularly recur in the division of all of its cells; and in all forms arising by sexual reproduction the number is even." This generalisation is still partially true but a number of types of numerical variation are now known to occur.

Numerical variation may involve whole chromosome sets (polyploidy) or a few chromosomes only (aneuploidy). Polyploidy is a balanced change involving no alteration of the numerical proportions of the chromosomes while aneuploidy, whether it involves loss or gain of chromosomes, leads to an unbalanced complement. In animals, which are generally diploids of high developmental complexity, aneuploidy is usually lethal. Trisomics often survive in diploid plants but they are frequently grossly deformed and sterile. In polyploid plants, however, aneuploidy is better tolerated since genotypic imbalance is buffered by the additional chromosome set(s).

In addition to intraspecific numerical variation, intra-individual polyploidy or aneuploidy occurs. In many diploids, production of polyploid cells is a regular feature of development, such as in the xylem elements of tomato (McMahon, 1956) and mammalian liver (Schwartz, 1956). Intra-individual aneuploidy is also known in both plants (e.g. *Claytonia virginica*, Lewis *et al.*, 1971) and animals. Nygren *et al.* (1968), for example, reported chromosome numbers varying from 18 to 75 in kidney cells of the pike ($2n = 50$).

In contrast to the general requirement for a balanced and usually constant number of A-chromosomes, B-chromosomes (supernumerary chromosomes)

may be present in some individuals of some natural populations. This gives a further source of numerical variation. B-chromosomes are additional to the members of the standard complement, are not homologous with them (the A-chromosomes), and usually differ from them in size, inheritance and lack of major phenotypic effects. B-chromosomes were first described by Longley (1927) and Randolph (1928) in maize and are now known in over 700 plant and animal species (Jones, 1975).

The nature and frequency of numerical variation in Scilla autumnalis in diploid (BB) populations from Corfu (Greece), autotetraploid (BBBB) populations from southern England, north-western France, the Channel Islands and Corfu and in autoallohexaploid (AABBBB) populations from England and the Channel Islands is considered in this chapter. Three types of numerical variation are reported: intra-individual variation (polysomaty and aneusomaty), inter-individual variation in the standard chromosomes (polyploidy and aneuploidy) and inter-individual variation in B-chromosomes.

## Results

### I. Numerical variation in diploid populations

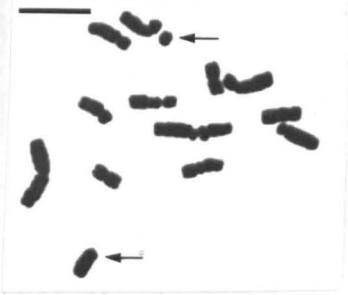
Eighty-one diploid plants of Scilla autumnalis from three populations on the island of Corfu, Greece, were examined. Only one plant deviated numerically from 2n = 14. This plant (PK21, Plate 4.1), from the Mt. Pantokrator population, had 15 chromosomes as a result of centric fission of chromosome 5 to give two telocentrics. This plant was also structurally variant with a short arm duplication in a chromosome 1 and an inversion in a chromosome 3.

### II. Numerical variation in tetraploid populations

#### a) Constant complements

Thirty-five tetraploid populations have been analysed and in twenty

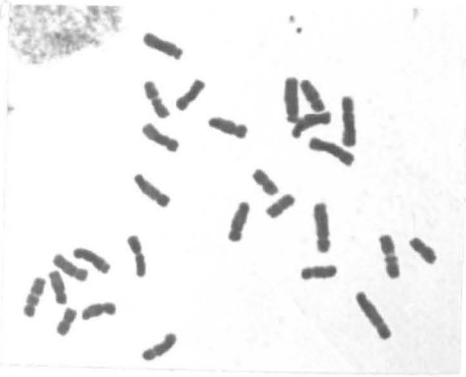
Plate 4.1 Numerical variation in diploid Scilla autumnalis



Centric fission heterozygote

Plate 4.2 Numerical variation in autotetraploid S. autumnalis

(a) 2n = 27 (-B4)

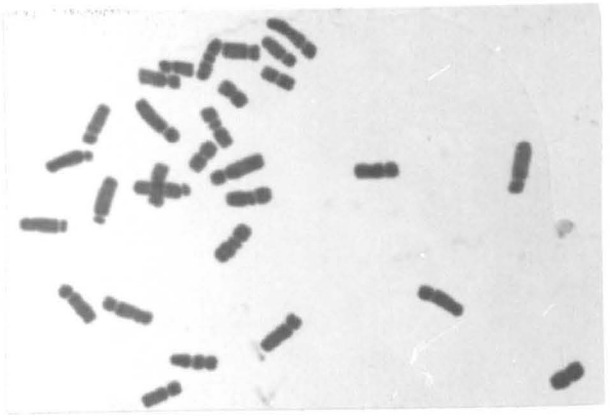


(b) 2n = 27 (-B6)

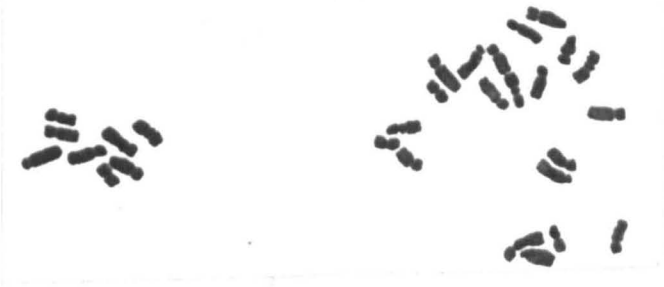
(c) 2n = 29 (+B1)

(d) 2n = 29 (+B2)

Plate 4.2 continued



(e) 2n = 29 (+B3)

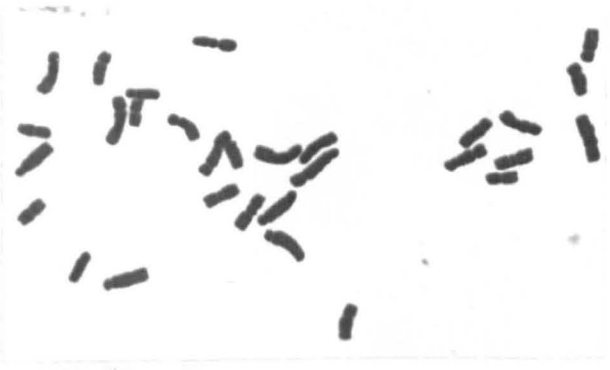


(f) 2n = 29 (+B4)

(g) 2n = 29 (+B5)

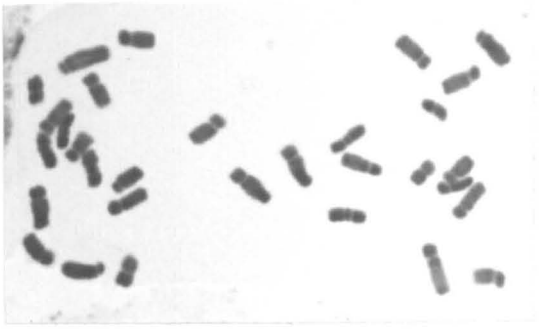
(h) 2n = 29 (+B6)

(i) 2n = 30 (+B4,B5)



(j) 2n = 30 (+B6,B7)

Plate 4.2 continued

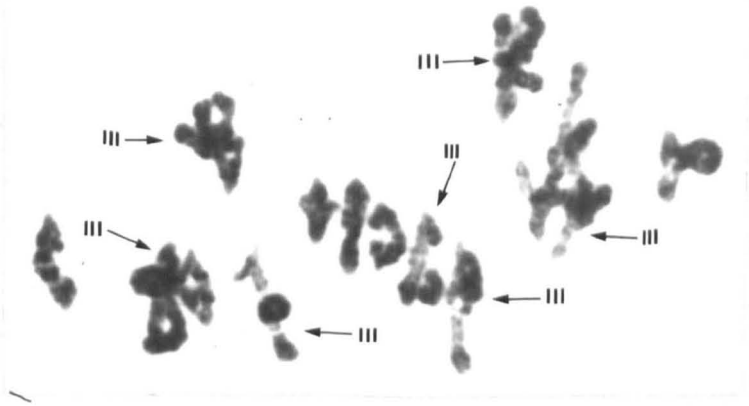


(k) 2n = 31 (+B1,B3,B6)

(l) 2n = 31 (+B1,B5,B6)

(m) 2n = 35 (+B1 - B7)

Plate 4.3 M-I in an autopentaploid (BBBBB)



of them at least one plant with a chromosome number deviating from 2n = 28 was found. These included both aneuploids and higher polyploids (Table 4.1).

The maximum number of deviant plants in a population was six out of 30 (20%) in the Fort Regent (Jersey) population (Table 4.1). More usually, however, a level of 3 to 6% of numerical variants is found. Overall, 39 plants out of 1163 were numerically variant (Table 4.1). Thirty of these were aneuploids (2.5%) of which 24 were pentasomics (2n = 29) and only six trisomics (2n = 27). If these aneuploids were generated by non-disjunction at meiotic anaphase with no differential survival of gametes or zygotes, then 27- and 29-chromosome plants would occur in populations with equal frequency. This is clearly not the case as there is a great deficiency of trisomics.

The chromosome lost or gained was identified in 29 of the 30 aneuploids (Table 4.2). The six trisomics were each missing a different chromosome (e.g. Plate 4.2 a,b). Only a chromosome 2 trisomic was not found. In the pentasomics, by contrast, eight of the 23 were pentasomic for chromosome 2 (e.g. Plate 4.2d). Chromosome 5 was the next most common excess chromosome (6 plants, e.g. Plate 4.2g) and all others of the haploid set were represented. The number of aneuploids, however, is too small for statistical inferences to be drawn.

Amongst these tetraploid populations four higher polyploid individuals were found (0.34%), two autohexaploids (BBBBBB, Fig. 3.2f) and two autopentaploids (BBBBB, Plate 4.2m). The autohexaploids presumably arose by fusion of a normal diploid gamete and an unreduced (tetraploid) gamete (Fig. 4.1). Autohexaploids, on backcrossing to autotetraploids, will produce pentaploid zygotes (Fig. 4.1). The two populations containing

Table 4.1 Chromosome numbers in tetraploid populations of Scilla autumnalis

| Population | Total Plants | Chromosome number | | | | | | | | Total plants deviating from 2n = 28* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Constant | | | | | | | Variable (28) | |
| | | 27 | 28 | 29 | 30 | 31 | 35 | 42 | | |
| MP | 28 | - | 27 | 1 | - | - | - | - | 1 | 1 |
| BN | 32 | - | 32 | - | - | - | - | - | - | 0 |
| PH | 30 | - | 30 | - | - | - | - | - | - | 0 |
| C+C | 34 | 1 | 33 | - | - | - | - | - | 2 | 1 |
| GP | 29 | - | 29 | - | - | - | - | - | 1 | 0 |
| GR | 32 | - | 32 | - | - | - | - | - | - | 0 |
| PP | 32 | - | 32 | - | - | - | - | - | 1 | 0 |
| IC | 33 | - | 31 | 2 | - | - | - | - | - | 2 |
| BH | 53 | - | 52 | 1 | - | - | - | - | 1 | 1 |
| LQP | 32 | - | 32 | - | - | - | - | - | - | 0 |
| IOW | 36 | - | 36 | - | - | - | - | - | - | 0 |
| HC | 30 | - | 30 | - | - | - | - | - | 1 | 0 |
| FC | 30 | - | 29 | 1 | - | - | - | - | - | 1 |
| GN | 30 | - | 30 | - | - | - | - | - | - | 0 |
| CB | 31 | - | 29 | 1 | 1 | - | - | - | - | 2 |
| FR | 30 | - | 24 | 4 | 1 | 1 | - | - | - | 6 |
| VP | 31 | - | 29 | 2 | - | - | - | - | - | 2 |
| LM | 7 | - | 7 | - | - | - | - | - | - | 0 |
| CC | 30 | - | 30 | - | - | - | - | - | - | 0 |
| SG | 151 | 4 | 143 | 2 | - | 1 | - | 1 | 3 | 8 |
| MG | 30 | - | 28 | 2 | - | - | - | - | - | 2 |
| LH | 27 | 1 | 25 | 1 | - | - | - | - | 1 | 2 |
| BSM | 31 | - | 31 | - | - | - | - | - | - | 0 |
| LV | 29 | - | 27 | 2 | - | - | - | - | 1 | 2 |
| PDG | 26 | - | 25 | 1 | - | - | - | - | - | 1 |
| PDT | 30 | - | 29 | 1 | - | - | - | - | - | 1 |
| PDP | 30 | - | 29 | - | 1 | - | - | - | - | 1 |
| PAL | 22 | - | 21 | - | - | - | 1 | - | - | 1 |
| PSG | 24 | - | 23 | - | - | - | 1 | - | - | 1 |
| SSL | 31 | - | 31 | - | - | - | - | - | - | 0 |
| ADC | 28 | - | 26 | 2 | - | - | - | - | - | 2 |
| PDA** | 36 | - | 36 | - | - | - | - | - | - | 0 |
| ASJ | 32 | - | 31 | 1 | - | - | - | - | 2 | 1 |
| AS | 23 | - | 22 | - | - | - | - | 1 | 1 | 1 |
| KV** | 23 | - | 23 | - | - | - | - | - | 1 | 0 |
| 35 pops. | 1163 | 6 | 1108 | 24 | 3 | 2 | 2 | 2 | 16 | 39 |
| | | 0.52% | 0.52% | 2.06% | 0.26% | 0.17% | 0.17% | | | 3.35% |

* (In the majority of cells)

** Populations with B-chromosomes.

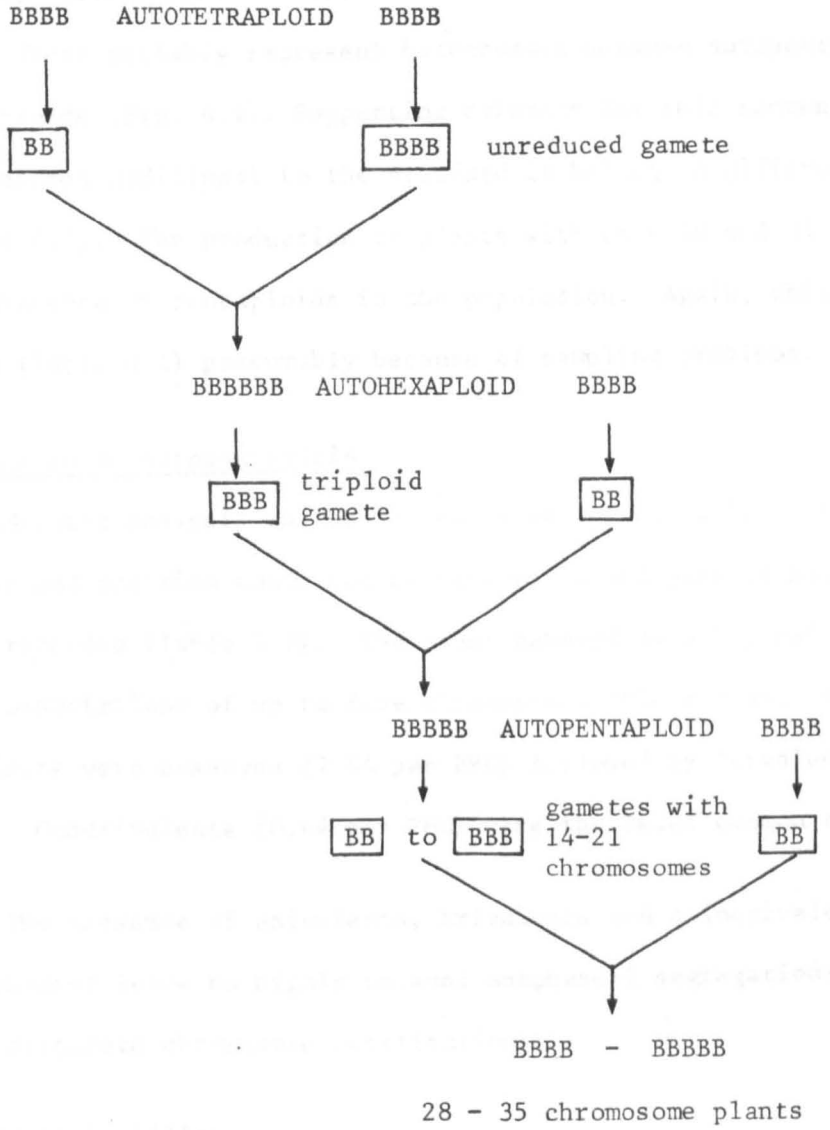
Table 4.2 The chromosomes lost and gained in trisomic and pentasomic plants from tetraploid populations of S. autumnalis

| Diploid number | Number of plants | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Chromosome lost or gained | | | | | | | Total plants |
| | B1 | B2 | B3 | B4 | B5 | B6 | B7 | |
| 27 | 1 | - | 1 | 1 | 1 | 1 | 1 | 6 |
| 29 | 1 | 8 | 1 | 3 | 6 | 2 | 2 | 23 |

Table 4.3 The additional chromosomes in 2n = 30 and 31 plants from tetraploid populations of S. autumnalis

| Diploid number | No. of plants | Additional Chromosomes |
|---|---|---|
| 30 | 3 | B1, B4; B4, B5; B6, B7 |
| 31 | 2 | B1, B3, B7; B1, B5, B6 |

Fig. 4.1 The role of nuclear restitution in the generation of numerical variation in tetraploid S. autumnalis



autopentaploids (PAL and PSG) should therefore, also contain the autohexaploids necessary for their production but in neither was this the case (Table 4.1). The small sample size (22 and 24 plants) and the low overall frequency of hexaploids (0.17%) presumably accounts for this.

The remaining numerical variants are those with 2n = 30 (three plants, 0.26%) Plate 4.2 i,j and 2n = 31 (two plants, 0.17%) Plate 4.2 k,l. These probably represent backcrosses between autopentaploids and tetraploids (Fig. 4.1). Supporting evidence for this contention is that chromosomes additional to the standard 28 belong to different groups (Table 4.3). The production of plants with 2n = 30 and 31 thus requires the presence of pentaploids in the population. Again, this has not been shown (Table 4.1) presumably because of sampling problems.

Meiosis in an autopentaploid

Meiotic analysis was performed in an autopentaploid (PSG 26). Chiasma number and position could not be determined and pairing patterns only were recorded (Table 3.7). The plant behaved as a typical autopentaploid with associations of up to five chromosomes (Plate 4.3). Most commonly bivalents were observed (7.44 per PMC) followed by trivalents (3.44 per PMC). Quadrivalents (0.44 per PMC) were the least common configuration.

The presence of univalents, trivalents and quinquivalents at metaphase-I leads to highly unequal anaphase-I segregations and gametes with disparate chromosome constitutions.

Pollen stainability

Pollen stainability of numerical variants was assessed using Alexander's differential stain (Alexander, 1969). The lowest pollen stainability was recorded for aneuploids (Table 4.4) and the highest for

Table 4.4 Pollen stainability and chromosome number in autotetraploid and autoallohexaploid races of S. autumnalis

| | 2n | Pollen stainability | | No. of plants |
|---|---|---|---|---|
| | | Mean (%) | s.d.(%) | |
| Tetraploids | 27 | 67.96 | 9.49 | 3 |
| | 28 | 72.16 | 15.28 | 64 |
| | 29 | 64.41 | 15.33 | 14 |
| | 30 | 68.58 | 12.79 | 3 |
| | 31 | 69.58 | 3.35 | 2 |
| | 35 | 73.05 | 15.86 | 2 |
| Hexaploids | 41 | 73.69 | 13.68 | 13 |
| | 43 | 77.62 | 10.14 | 24 |
| | 43 | 80.47 | 14.52 | 7 |

Table 4.5 Analysis of variance of pollen stainability in plants with different chromosome numbers from tetraploid populations (after angular transformation)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between karyotypes | 5 | 739.305 | 147.861 | 0.653 | >0.2 |
| Between plants | 82 | 18570.445 | 226.469 | | |
| Total | 87 | 19309.750 | | | |

tetraploids and pentaploids. The standard deviations were very high, however, and these differences were not significant ($F = 0.653$, $P > 0.2$; Table 4.5).

b) Variable complements

The vast majority of the 1163 plants from tetraploid populations were karyotypically constant in root tip cells. However, in 16 plants (1.4%) (from 12 different populations) one or two root tip cells showed aberrant chromosome numbers as a result of aneuploidy or polyploidy (Table 4.7). In most cases, only one or two cells of the approximately ten in two roots scored deviated numerically by gain or loss of a chromosome (aneusomaty) or by the process of spontaneous non-reduction (polysomaty) (Plate 4.4).

Three classes of numerically variable plants were identified (Table 4.7). The first class contains a single plant (GP 27) in which the two roots were constant but of different chromosome numbers ($2n = 27$, and $2n = 28$). This plant is probably basically $2n = 28$ but chromosome loss occurred immediately prior to root initiation to give at least two cell lines in the roots.

The plants in the second class (12) have one uniform and one mosaic root. Eight plants were aneuploid mosaics. In six, one root was constant with $2n = 28$ and the other variable with 1 or 2 cells with $2n = 29$; in two plants the variant cells were $2n = 27$. Non-disjunction at anaphase has clearly occurred after root initiation. The remaining four plants illustrate the occurrence of sporadic nuclear restitution with mosaic $2n = 28 + 56$ roots.

In the third, rather heterogeneous, class both roots studied were variable. In plant C + C18, both roots contained a single octoploid cell with 56 chromosomes (Plate 4.4a). Plant HC21 was of the constitution $2n = 28/27, 28/29$ with one deviant cell in each root. ASJ1 was the most

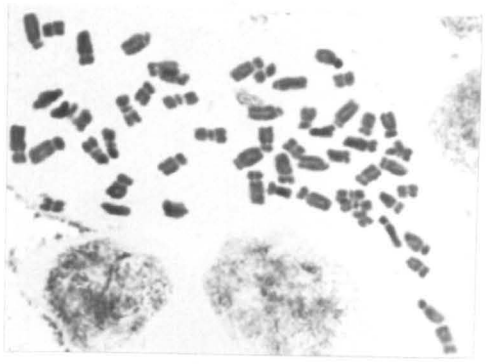
Table 4.6 Analysis of variance of pollen stainability in plants with different chromosome numbers from hexaploid populations (after angular transformation)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between karyotypes | 2 | 235.509 | 117.754 | 0.823 | >0.2 |
| Between plants | 41 | 5867.063 | 143.099 | | |
| Total | 43 | 6102.572 | | | |

Table 4.7 The types of numerically variable plants in tetraploid populations of S. autumnalis (two roots scored per plant)

| Type of numerical variation | Numbers of plants | | | | | | | | | | | | Total plants |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | C+C | GP | PP | BH | HC | SG | LH | LV | ASJ | AS | KV | |
| Both roots constant | | | | | | | | | | | | | |
| 2n = 28/27 | - | - | 1 | - | - | - | - | - | - | - | - | - | 1 |
| One constant and one variable root | | | | | | | | | | | | | |
| 2n = 28, 28/27 | - | - | - | - | - | - | 2 | - | - | - | - | - | 2 |
| 28, 28/29 | 1 | - | - | - | 1 | - | 1 | - | 1 | 1 | 1 | - | 6 |
| 28, 28/56 | - | 1 | - | 1 | - | - | - | 1 | - | - | - | 1 | 4 |
| Both roots variable | | | | | | | | | | | | | |
| 2n = 28/27, 28/29 | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 |
| 27/28/29, 28/27 | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 |
| 28/56, 28/56 | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 |
| Total | 1 | 2 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 1 | 16 |

Plate 4.4 Polysomaty and extreme aneusomaty in autotetraploid *Scilla autumnalis*



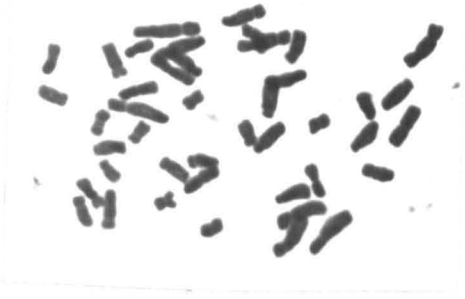
(a) Polysomaty
2n = 56

(b) Aneusomaty
2n = 24

Plate 4.5 Numerical variation in autoallohexaploid *Scilla autumnalis*

(a) 2n = 41 (-A7)

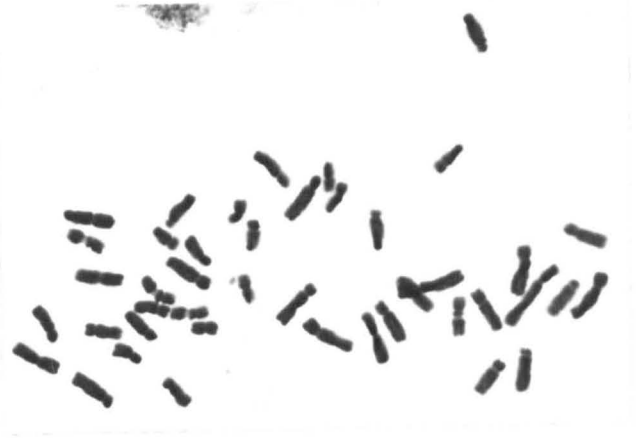
(b) 2n = 41 (-B2)



(c) 2n = 43 (+A3)

Plate 4.5 continued

(d) 2n = 43 (+A7)



(e) 2n = 43 (+B2)

(f) 2n = 43 (+B4)

numerically variable tetraploid found, exhibiting both between and within root variability. One root contained cells with 2n = 27, 28 and 29 with 2n = 27 the majority class (Table 4.8). By contrast, the other root was predominantly 2n = 28 with two cells of 2n = 27. ASJ1, then, shows inherent instability of chromosome number.

III. Numerical variation in hexaploid populations

a) Constant complements

In the autoallohexaploids only 188 of the 264 plants scored (76.4%) showed constancy of chromosome number (Table 4.9) and of these 27 were aneuploids, comprising 15 plants with 2n = 41 (6.1%) and 12 plants with 2n = 43 (4.9%) (e.g. Plate 4.5 ). No plants with ploidy levels higher than 6x were found.

b) Variable complements

Remarkably, chromosomally variable plants, having at least one deviant complement, were even more common than constant numerical variants (58 out of 246; Table 4.9). The 58 variable plants included 22 plants with the most frequent chromosome number other than 2n = 42. That is, they were variable aneuploids, and of these, all but one, which had 2n = 40, were 2n = 41 and 43 individuals.

The variable plants can again be subdivided into three classes (Table 4.11): (i) seven plants with both roots constant but distinct in chromosome number; (ii) forty three plants with one root constant and one variable and (iii) eight plants with both roots variable.

(i) The most common type in the first class contained 2n = 41, 42 (4 plants), followed by 2n = 42, 43 (2 plants) and a single extreme plant with 2n = 42, 45.

Table 4.8 Numerical chromosome variation in two roots of the tetraploid plant ASJ1 (50 cells scored in each root)

| Chromosome number | Number of cells | |
|---|---|---|
| | Root 1 | Root 2 |
| 27 | 43 | 2 |
| 28 | 2 | 48 |
| 29 | 5 | - |

Table 4.9 Chromosome numbers in hexaploid populations of S. autumnalis. Variable plants are classified according to the chromosome number found in the majority of cells.

| Population | Total Plants | Chromosome number | | | | | | | | | Total plants deviating from 2n = 42 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Constant plants | | | | Variable plants | | | | | |
| | | 41 | 42 | 43 | total | 40 | 41 | 42 | 43 | total | |
| CP | 39 | 1 | 29 | - | 30 (88.2%) | - | 1 | 1 | 2 | 4 (11.8%) | 4 (11.8%) |
| CT | 30 | 3 | 15 | 3 | 21 (70.0%) | - | - | 7 | 2 | 9 (30.0%) | 8 (26.7%) |
| GD | 31 | 1 | 15 | 2 | 18 (58.1%) | - | 2 | 8 | 3 | 13 (41.9%) | 8 (25.8%) |
| RT | 35 | 2 | 22 | 3 | 27 (77.1%) | - | 1 | 6 | 1 | 8 (22.9%) | 7 (20.0%) |
| LC | 28 | 2 | 19 | 1 | 22 (78.6%) | - | 1 | 3 | 2 | 6 (21.4%) | 6 (21.4%) |
| LE | 28 | 2 | 19 | - | 21 (75.0%) | - | 1 | 5 | 1 | 7 (25.0%) | 4 (14.3%) |
| SMP | 31 | 1 | 21 | 1 | 23 (74.2%) | 1 | - | 5 | 2 | 8 (25.8%) | 5 (16.1%) |
| LL | 29 | 3 | 21 | 2 | 26 (89.7%) | - | - | 1 | - | 3 (10.3%) | 7 (24.1%) |
| Total Plants | 246 | 15 (6.1%) | 161 (65.4%) | 12 (4.9%) | 188 (76.4%) | 1 (0.4%) | 8 (3.3%) | 36 (14.6%) | 13 (5.3%) | 58 (23.6%) | 49 (19.9%) |

Table 4.10 Summary of chromosome numbers in hexaploid populations of S. autumnalis. (Variable plants have been classified according to the chromosome number found in the majority of cells)

| Chromosome number | 40 | 41 | 42 | 43 | Total plants |
|---|---|---|---|---|---|
| No. of plants | 1 (0.41%) | 23 (9.35%) | 197 (80.08%) | 25 (10.17%) | 246 |

Table 4.11 The types of numerically variable plants in hexaploid populations of S. autumnalis (five cells from each root)

| Type of numerical variation | Number of plants | | | | | | | | Total plants |
|---|---|---|---|---|---|---|---|---|---|
| | Population | | | | | | | | |
| | CP | CT | GD | RT | LC | LE | SMP | LL | |
| Both roots constant | | | | | | | | | |
| 2n = 41,42 | 1 | - | 1 | - | 1 | - | 1 | - | 4 |
| 42,43 | - | - | 1 | - | 1 | - | - | - | 2 |
| 42,45 | - | - | - | 1 | - | - | - | - | 1 |
| Sub-total | 1 | 0 | 2 | 1 | 2 | 0 | 1 | 0 | 7 |
| One constant and one variable root | | | | | | | | | |
| 2n = 41,41/40 | - | - | - | - | - | 1 | - | - | 1 |
| 41,41/40/42 | - | - | - | - | 1 | - | - | - | 1 |
| 41,41/42 | 1 | - | 1 | 1 | 1 | - | - | 2 | 6 |
| 41,43/44 | - | - | 1 | - | - | - | - | - | 1 |
| 42,42/41 | - | 2 | 1 | 1 | - | - | 1 | 1 | 6 |
| 42,42/43 | - | 1 | 4 | 1 | - | 3 | 2 | - | 11 |
| 42,42/44 | - | 1 | 1 | - | - | - | - | - | 2 |
| 42,42/84 | - | 1 | - | - | - | 1 | - | - | 2 |
| 42,42/43/41 | - | - | - | 1 | - | - | - | - | 1 |
| 42,42/45 | - | - | - | 1 | - | - | - | - | 1 |
| 42,42/41/40 | - | - | 1 | - | - | - | - | - | 1 |
| 42,43/44 | - | 1 | - | - | - | - | - | - | 1 |
| 43,43/42 | - | - | 1 | 1 | - | - | 1 | - | 3 |
| 43,43/42/44 | - | 1 | - | - | - | 1 | - | - | 2 |
| 43,41/43 | 1 | - | - | - | - | - | - | - | 1 |
| 43,43/44 | 1 | - | - | - | - | - | - | - | 1 |
| 43,42/43 | - | 1 | - | - | 1 | - | - | - | 2 |
| Sub-total | 3 | 8 | 10 | 6 | 3 | 6 | 4 | 3 | 43 |
| Both roots variable | | | | | | | | | |
| 2n = 40/41,41/40 | - | - | - | - | - | - | 1 | - | 1 |
| 42/41,42/41 | - | - | - | - | - | - | 1 | - | 1 |
| 42/41,42/43/41 | - | - | - | - | - | 1 | - | - | 1 |
| 42/43,42/43 | - | - | 1 | 1 | - | - | - | - | 2 |
| 42/43,43/42 | - | 1 | - | - | - | - | - | - | 1 |
| 43/44,43/42 | - | - | - | - | - | - | 1 | - | 1 |
| 43/44,43/44 | - | - | - | - | 1 | - | - | - | 1 |
| Sub-total | 0 | 1 | 1 | 1 | 1 | 1 | 3 | 0 | 8 |
| Total plants | 4 | 9 | 13 | 8 | 6 | 7 | 8 | 3 | 58 |

(ii) In the second class of 43 plants, seventeen different combinations were distinguished. Three combinations were particularly frequent: 2n = 42, 42/43 (11 plants), 2n = 42, 42/41 (6 plants) and 2n = 41, 41/42 (6 plants). The remaining fourteen combinations involved less than four plants each.

(iii) Seven distinct types were recorded with no more than two plants in any one class.

More detailed analyses of variable plants (Table 4.12) showed the same trends although the extent of the variability uncovered increased with numbers of roots examined. GD10, for example, contained cells with from 41 to 44 chromosomes in a sample of eight roots.

Approximately equal numbers of both variable and constant aneuploids with 2n = 41 and 2n = 43 were found (23:25; Table 4.10) in contrast to tri- and pentasomic tetraploids. The total number of aneuploids, including constant and variable plants, was 58 (23.6%) which is indicative of the gross instability of the hexaploid complement.

In 38 of the 49 aneuploids the chromosome involved has been identified (Table 4.13). A slight excess involvement of the B genome is indicated (17A: 23B). It would be expected, however, that B genome chromosomes would be involved in aneuploid changes twice as frequently as A genome chromosomes. The deviation from expected is not significant ($\chi^2 = 1.54$, $P > 0.2$; Table 4.14). Similarly, all chromosomes within a genome are involved in loss or gain although B2 appears the most susceptible (e.g.Plate 4.5 ).

Pollen stainability

Interestingly, no significant difference in stainability of pollen from 2n = 41, 42 and 43 plants was detected ($F = 0.823$, $P > 0.2$; (Table 4.6).

Table 4.12 Numerical chromosome variation in four hexaploids.

(Five cells scored in each root)

| Plant | Number of roots scored | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chromosome number | | | | | | Total roots |
| | 41 | 42 | 43 | 44 | 42/43 | 43/44 | |
| CT4 | - | 2 | 15 | 1 | - | 2 | 20 |
| CT6 | - | 2 | 3 | - | 1 | - | 6 |
| CT25 | - | - | 1 | - | 4 | - | 5 |
| GD10 | 3 | - | 4 | - | - | 1 | 8 |

Table 4.13 The chromosomes lost and gained in aneuploid plants from hexaploid populations of S. autumnalis

| 2n = | Number of plants | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chromosome lost or gained | | | | | | | | | | | | | | | Total plants |
| | A genome | | | | | | Sub-total | B genome | | | | | | | Sub-total | |
| | A1 | A2 | A3 | A4-A5 | A6 | A7 | | B1 | B2 | B3 | B4 | B5 | B6 | B7 | | |
| 41 | 1* | - | 1 | 2* | 2 | 5 | 11 | 1 | 5 | 3 | 1 | - | 1 | 1 | 12 | 21 |
| 43 | - | 2 | - | 2 | - | 1 | 6 | 3 | 5 | - | 1 | 1 | 1 | - | 11 | 17 |

* 1 plant with 2n = 40 (chromosomes lost: A1, A5).

IV. Numerical variation and ploidy

The incidence of numerical variation increases dramatically with ploidy level. For constant numerical mutants the level rises from 3.4% to tetraploids to 10.98% in hexaploids (Table 4.15). Variable numerical mutants increase even more from 1.38% to 23.5% of plants (Table 4.16). A buffering effect of the increased number of genomes on numerically abnormal complements is clearly evident. At the diploid level, aneuploidy may well be lethal and indeed the only numerical variant observed resulted from centric fission which does not alter the genic balance.

Polysomaty (somatic non-reduction) is clearly an infrequent occurrence and incidence does not seem to be related to ploidy level (0.05% of cells in tetraploids, 0.08% in hexaploids; Table 4.17).

V. B-chromosomes

B-chromosomes have been found in two tetraploid populations: Pont de l'Argenton in France (PDA) and Kavos in Corfu (KV). The morphology of the Bs was quite distinct.

a) Pont de l'Argenton

(i) Types of B

Four different euchromatic B-chromosomes were found in this population; three sub-telocentrics and a metacentric (Fig. 4.2; Plate 4.6 a-i). The three subtelocentric Bs have the same length short arms (0.25 μm) but differ in their long arms. $B^{st-1}$ is 4.43 μm in length, $B^{st-2}$ 3.45 μm and $B^{st-3}$ 2.46 μm. The metacentric $B^{m}$, 8.36 μm in length, is perhaps an isochromosome derivative of the long arm of $B^{st-1}$.

The most common Bs were $B^{st-2}$ and $B^{st-1}$ and plants carrying both types have been found (Table 4.18; Plate 4.6 d,g). Homologies between these B-types

Table 4.14 Observed and expected numbers of A and B genome chromosome losses and gains in autoallohexaploid plants (expected numbers calculated on the assumption that B genome chromosomes will be lost twice as frequently as A genome chromosomes)

| No. of chromosomes lost/gained | A genome | B genome | Total |
|---|---|---|---|
| observed | 17 | 23 | 40 |
| expected | 13.33 | 26.67 | 40 |

$\chi^2 = 1.54$ ($P > 0.2$)

Table 4.15 The incidence of numerical variation in the chromosome races of S. autumnalis (constant plants)

| Chromosome race | Total plants | No. of numerical variants |
|---|---|---|
| BB (2x) | 81 | 1 (0.22%) |
| BBBB (4x) | 1163 | 39 (3.35%) |
| AABBBB (6x) | 246 | 27 (10.98%) |

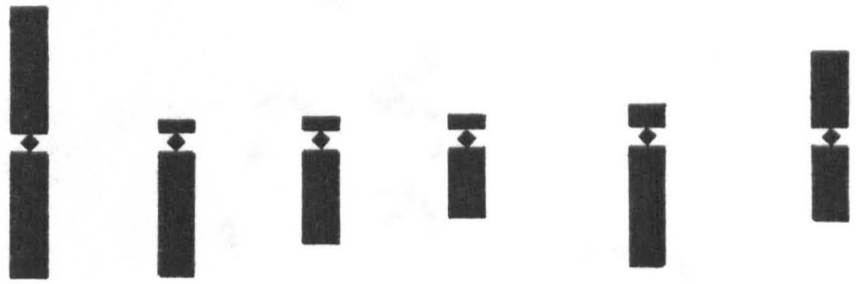
Table 4.16 The numbers of numerically variable plants in autotetraploid and autoallohexaploid races of S. autumnalis

| Race | Total plants | No. of numerical variants | | | Total |
|---|---|---|---|---|---|
| | | Both roots constant | One constant and one variable root | Both roots variable | |
| BBBB (4x) | 1163 | 1 | 12 | 3 | 16 (1.38%) |
| AABBBB (6x) | 246 | 7 | 43 | 8 | 58 (23.58%) |

Table 4.17 The incidence of polysomaty in the chromosome races of S. autumnalis

| Chromosome race | Total cells scored | No. of polysomic cells |
|---|---|---|
| BB (2x) | 668 | - |
| BBBB (4x) | 11169 | 6 (0.54%) |
| AABBBB (6x) | 2543 | 2 (0.079%) |

Fig. 4.2 The morphology of B-chromosomes in two populations of tetraploid S. autumnalis. A B7 chromosome is included as a reference




| | $B^m$ | $B^{st-1}$ | $B^{st-2}$ | $B^{st-3}$ | $B^{st-4}$ | B7 |
|---|---|---|---|---|---|---|
| Short arm(μm) | 4.18 | 0.25 | 0.25 | 0.25 | 0.74 | 4.92 |
| Long arm (μm) | 4.18 | 4.18 | 3.20 | 2.21 | 4.18 | 4.92 |
| Total (μm) | 8.36 | 4.43 | 3.45 | 2.46 | 4.92 | 9.84 |
| Arm ratio | 1:1.0 | 1:16.7 | 1:12.8 | 1:8.8 | 1:5.6 | 1:1.0 |

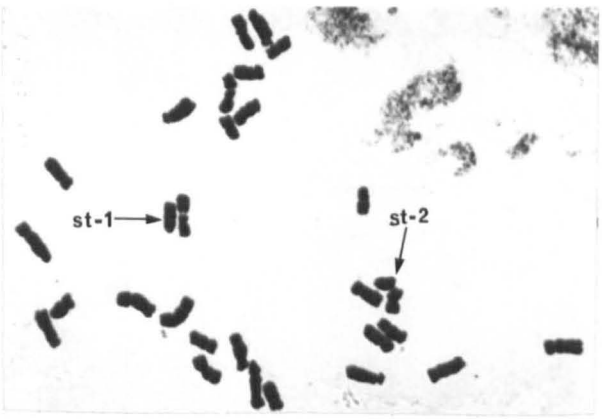
Plate 4.6 B-karyotypes of tetraploid Scilla autumnalis

a - i Pont de l'Argenton

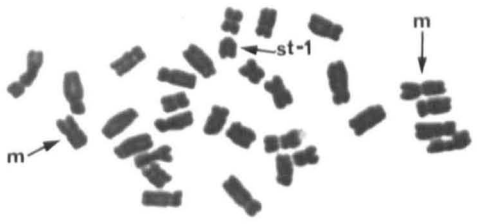
(a) $B^{st-2}$

(b) $2B^{st-1}$

(c) $2B^{st-2}$




(d) $B^{st-1} + B^{st-2}$

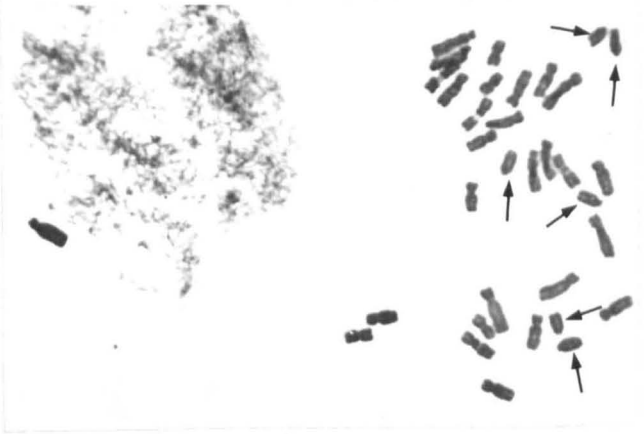



(e) $B^{st-1} + 2B^{m}$

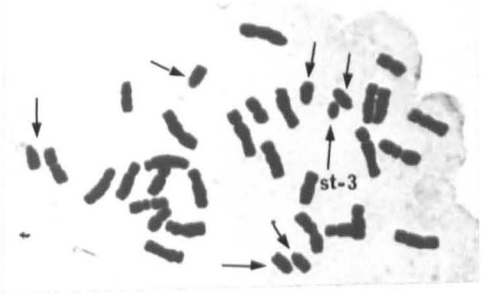
Plate 4.6 continued

(f) $4B^{st-2}$


(g) $2B^{st-1} + 2B^{st-2}$



(h) $6B^{st-1}$




(i) $6B^{st-2} + B^{st-3}$

(j) Kavos B

Table 4.18 B-constitution of thirty six Scilla autumnalis plants from the tetraploid population PDA

| B-number | B-karyotype | No. of plants | |
|---|---|---|---|
| 1 | 1 st-1 | 2 | 6 |
| | 1 st-2 | 4 | |
| 2 | 2 st-1 | 4 | 10 |
| | 2 st-2 | 3 | |
| | 1 st-1 + 1 st-2 | 3 | |
| 3 | 1 st-1 + 2 m | 1 | |
| 4 | 4 st-2 | 1 | 2 |
| | 2 st-1 + 2 st-2 | 1 | |
| 6 | 6 st-1 | 1 | |
| 7 | 6 st-2 + 1 st-3 | 1 | |

Table 4.19 Summary of the B-constitution of plants from the tetraploid population PDA

| | |
|---|---|
| Total plants | 36 |
| No. of B-types | 4 |
| No. of B-karyotypes | 10 |
| No. of B-containing plants | 21 |
| % B-containing plants | 58.3 |
| Mean no. Bs/plant | 1.39 |

have been deduced from meiotic analysis of such plants (see below). The single $B^{st-3}$ found was present in a plant which also contained 6 $B^{st-2}$ (Plate 4.6i). This is presumably a deletion product of one of the other subtelocentrics but no meiotic analysis has yet been attempted. The $B^{m}$ was found in one plant which contained $2B^{m}$s and one $B^{st-1}$ (Plate 4.6e).

(ii) Numbers of Bs

The average number of Bs per plant was 1.39 (Table 4.19). Most commonly B-containing individuals have 2Bs (10 plants) or 1B (6 plants) but plants with up to 7 Bs (Plate 4.6i) have been found (Fig. 4.3). Ten morphologically and numerically distinct B complements were found in this population sample (Table 4.18).

If B-chromosomes were distributed at random in the population, then a Poisson distribution would be expected. This is not the case, due to an excess of even numbered complements ($\chi^2_{(2)} = 7.73$, $P < 0.05$; Table 4.20).

(iii) Meiotic behaviour of B-chromosomes

The meiotic behaviour of B-chromosomes was examined in PDA6, a plant with $2B^{st-1} + 2B^{st-2}$ (Tables 4.21, 4.22).

Chiasma formation between B-chromosomes is much less efficient that between A-chromosomes with a chiasma frequency of 0.74 per B-bivalent. Metaphase-I pairing was not restricted to similar-sized Bs but pairing was markedly preferential; homomorphic bivalents were twice as frequent as heteromorphic bivalents (Plate 4.7 ; Table 4.21).

Most B-bivalents had a single chiasma in the long arm but bivalents with two long arm chiasmata were seen (Plate 4.7c). No B-quadrivalents or trivalents were found, however, despite a sufficiency of B-chiasmata in some PMCs. The most common PMC class contained two B-bivalents (39%)

Fig. 4.3 The frequency of plants with different numbers of B-chromosomes in the tetraploid population PDA

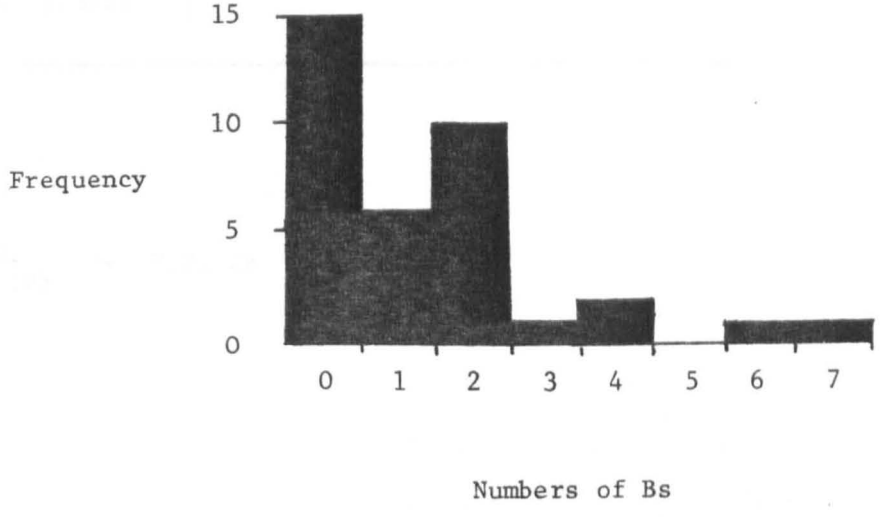


Table 4.20 Observed and expected frequencies of plants with different numbers of B-chromosomes in the tetraploid population PDA (expected frequencies calculated on the basis of the Poisson distribution)

| No. Bs | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Total |
|---|---|---|---|---|---|---|---|---|---|
| No. plants | 15 | 6 | 10 | 1 | 2 | 0 | 1 | 1 | 36 |
| Expected no. plants | 8.98 | 12.47 | 8.66 | 5.89 | | | | | 36 |

$\chi^2_{(2)} = 7.73 \ (P < 0.05)$

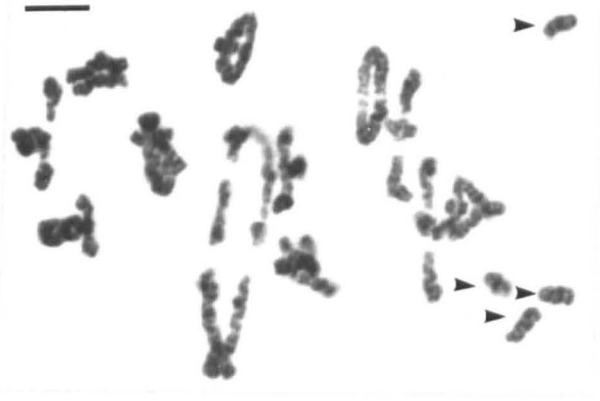
Table 4.21 B-chromosome pairing patterns at metaphase-I in a tetraploid plant (PDA6) with four B-chromosomes ($2B^{st-1} + 2B^{st-2}$). PMCs with two B-bivalents were not classified into heteromorphic or homomorphic bivalents

| Pairing pattern | No. cells | | Total PMCs |
|---|---|---|---|
| | Bivalent type | | |
| | Heteromorphic ($B^{st-1} + B^{st-2}$) | Homomorphic ($2B^{st-1}$ or $2B^{st-2}$) | |
| 4I | - | - | 8 |
| II + 2I | 12 | 24 | 36 |
| 2II | 56 | | 56 |
| Total | - | - - | 100 |

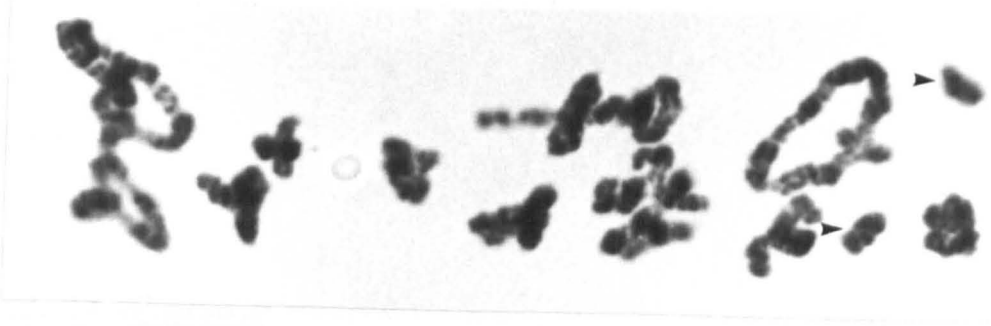
Table 4.22 B-chromosome chiasma number variation in a tetraploid plant (PDA6) with four B-chromosomes ($2B^{st-1} + 2B^{st-2}$)

| Chiasma No. per PMC | No. PMCs |
|---|---|
| 0 | 8 |
| 1 | 3 |
| 2 | 39 |
| 3 | 19 |
| 4 | 1 |
| Total PMCs | 100 |

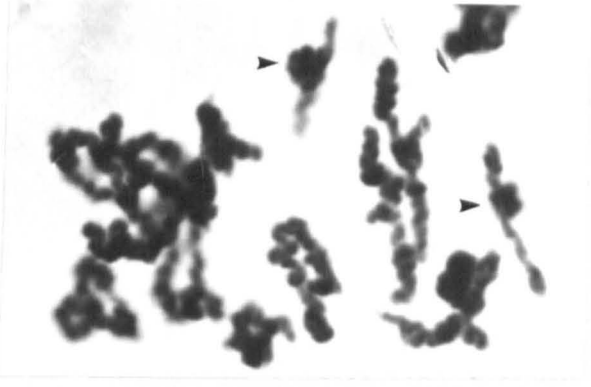
Plate 4.7 Meiosis in an autotetraploid (PDA6) with 4 B-chromosomes ($2B^{st-1} + 2B^{st-2}$)



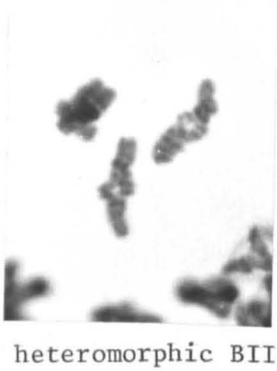
(a) 4BIs



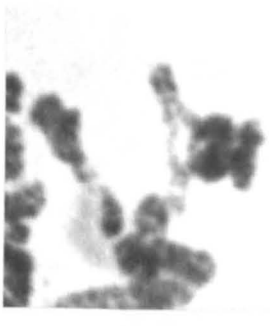
(b) BII + 2BIs



(c) Two and one chiasmate BIIs



(d) Two heteromorphic BIIs



(e) Proximal reverse chiasma in a BII

followed by those with a single bivalent and two univalents (33%; Plate 4.7 a-d).

Clearly $B^{st-1}$ and $B^{st-2}$ are partially homologous and the two may be related by a simple deletion/duplication difference. However, it is possible that a more complex relationship exists. A two-chiasmate bivalent was observed with a proximal reverse chiasma indicating either a paracentric inversion loop or a spontaneous U-type exchange (Plate 4.7e).

b) Kavos, Corfu

The B-chromosome from Kavos was 4.92 μm in length with an arm ratio of 1:5.65 (Fig. 4.2; Plate 4.6j). These euchromatic Bs were found in three plants in the population sample of 23.

## Discussion

### I. Aneuploidy

In Scilla autumnalis aneuploidy is rare in diploids (0.23%) but more frequent in tetraploids (3.35%) and particularly hexaploids (10.98%).

Aneuploidy is rare in natural populations of diploid organisms, particularly so in animals, since such chromosomal imbalance is usually inimical to the development of the individual (Darlington, 1965; John and Lewis, 1968). In man, although chromosomal aneuploidies contribute significantly to foetal wastage, a survey of 13,751 consecutive births (Walzer and Gerald, 1977) included only 19 autosomal aneuploidies (0.14%). In spontaneous abortuses the frequency of autosomal aneuploidy is very much higher, with 41% of aneuploids in 1498 abortuses studied by Boué et al. (1975).

Data on the frequency of aneuploidy in diploid plants is scarce. Navashin (1926), in large scale surveys of Crepis capillaris and C. tectorum,

found aneuploids at a frequency of 0.45% and 0% respectively.

The increase in the frequency of aneuploidy with ploidy level in S. autumnalis emphasises that tetraploids are better able to tolerate chromosomal loss or gain because of the buffering effect of multiple genomes; hexaploids are even more highly buffered. In Triticum aestivum, for example, even nullisomics survive and may set some seed (Sears, 1953). In tetraploid S. autumnalis, as in human abortuses, chromosome loss is less frequently detected than gain.

Aneuploids are frequent in apomictic species (which are often higher polyploids e.g. Poa alpina; Gustafsson, 1947) but extensive aneuploid variation amongst wild sexual polyploids as in S. autumnalis (where there is no evidence for vegetative spread) is rare. Some of the few species showing such variation are Erophila verna (Winge, 1940), Cardamine pratensis (Urbanska Worytkiewicz and Landolt, 1974), Hierochlöe alpina (Weimarck, 1976), Claytonia virginica (Lewis, 1962; Lewis et al. 1967), Narcissus bulbocodium (Darlington, 1963) and Koeleria vallesiana (Callow, 1977).

A similar relationship with ploidy level to that in S. autumnalis has been described for Elymus (Heneen, 1977a,b; Heneen and Runemarck, 1977) where the degree of aneuploidy increases between the diploid E. striatulus and the octoploid E. diae, the tetraploid E. rechingeri being intermediate.

Genomic buffering may well be responsible for the survival of aneuploids in polyploid complexes but the extremely high frequency of aneuploidy amongst hexaploids of S. autumnalis may in part be caused by higher rates of meiotic error at this ploidy level. In tetraploid Dactylis glomerata aneuploids are produced sexually and account for 10-40% of seed

(Müntzing, 1937; Myers and Hill, 1940) although only 3.2% of wild plants are aneuploid (Jones, 1962). In Agrostis stolonifera and Holcus mollis, seedling progeny from pentaploid and hexaploid races are mainly aneuploid although in natural populations few aneuploids occur (Jones, 1957, 1958).

In Lolium perenne the three main causes of the production of gametes were unequal segregation from quadrivalents at AI, irregular disjunction of quadrivalents at AI leading to laggards and unpaired chromosomes at MI (Myers, 1945). A later study by Simenson (1973) indicated that aneuploid gametes resulted from indifferent and linear quadrivalent orientation at MI. This may also be the case in tetraploid Scilla autumnalis in which univalents and trivalents are rare at metaphase-I. By contrast, aneuploids in tetraploid rye have been ascribed to extensive trivalent and univalent formation (Hazarika and Rees, 1967).

II. Aneusomaty

Plants exhibiting aneusomaty (variable plants) occur in about a quarter of the tetraploid populations and all hexaploid populations. In the latter race, the incidence of aneusomaty was relatively constant, and remarkably high in the populations, with about 20% of plants exhibiting the phenomenon.

Aneusomaty was first described in Caladium bicolor (Sharma and Das, 1954) where chromosome number in the shoot apex was found to be variable. Aneusomaty has been noted in a number of other plant species and in Hymenocallis calathinum aneusomaty appears to be the rule, for no constant chromosome number can be found in root tips (Snoad, 1955). This is also the case in PMCs although the range of chromosome numbers is less. In another Scilla species, S. scilloides (a polyploid complex), aneuploid cells occur only with a frequency of 0.54% in root tips (Noda, 1975).

Aneusomaty may be more prevalent in polyploid than diploid plants for mechanical reasons, since there are more chromosomes to interfere with the regularity of the mitotic process and induce non-disjunction. Sharma (1974) has proposed that aneusomaty is restricted to partly or wholly asexually-reproducing species, in which it may play a role in generating diversity. This hypothesis must be questioned in the light of the occurrence of aneusomaty in S. autumnalis, which reproduces strictly by sexual means.

III. Polysomaty

Polysomaty is probably a constant but sporadic phenomenon in plants and has been noted in the related Scilla species S. siberica (Noda, 1975) and also in Allium schoenoprasum (Bougourd, 1977). In Allium schoenoprasum, occasional tetraploid roots were found in basically diploid individuals and, more commonly, a few tetraploid cells were found to occur in otherwise diploid roots.

Instances of polysomaty in somatic tissues of S. autumnalis are very rare both in autotetraploids (giving octoploid cells) and autoallohexaploids (giving duodecaploid cells). No cases of polysomaty have yet been found in the small sample of diploid cells.

Non-reduction at meiosis in Scilla autumnalis must also occur since hexaploids and pentaploids are found in predominantly tetraploid populations. In diploid and hexaploid populations, however, no higher polyploids were found. In hexaploid populations of Koeleria vallesiana, by contrast, heptaploid (7x) and enneaploid (9x) plants have been found (Callow, 1977).

IV. B-chromosomes

B-chromosomes have been reported in diploid Scilla autumnalis from Spain (Ruiz Rejón et al., 1980), Sicily (Battaglia, 1963) and Palestine

(Battaglia, 1964) but have not previously been detected in tetraploids. The B-chromosomes reported in this thesis were both from tetraploid populations and are euchromatic in contrast to those in Spanish diploids which are highly heterochromatic (Ruiz Rejón et al., 1980).

The mean frequency of Bs in the PDA population was high (1.38 per plant) in comparison to both the KV population (0.13 per plant) and the diploid population (0.15 per plant; Ruiz Rejón, 1980).

In many plant species the distribution of individuals with different numbers of Bs fits a Poisson distribution. For example the B distribution in a diploid population from Spain conformed to a Poisson (data of Ruiz Rejón, 1980). This was not the case in the tetraploid PDA population where there is a great excess of 2B individuals in the sample and some indication of a 4B excess. The excess of even B-numbers may suggest a selective advantage or, perhaps more likely, may be a reflection of the mechanism of inheritance. Such a distribution is characteristic of the B-systems in Gramineae such as Secale (Rees and Jones, 1977) resulting from polarised non-disjunction at PGMI.

In all tetraploid B-containing individuals of Scilla autumnalis, the Bs were mitotically stable in root tips, even in the plant with seven Bs. By contrast, a single diploid plant from Palestine was described by Battaglia (1964) as having a variable B-complement between 6 and 8 Bs.

Four different types of B were found in the tetraploid PDA population. It is likely that these different types of B are related, and have been produced by deletion and isochromosome formation from an original B. Bs are in general less stable than A-chromosomes in inheritance and are known in many species to undergo misdivision of the centromere to produce isochromosomes.

Because Bs are non-essential, the derived Bs may persist in the population and undergo further structural rearrangement. Examples of complex derivative Bs are found in Allium schoenoprasum, Liliaceae (Bougourd and Parker, 1979) and Aster ageratoides, Compositae (Matsuda, 1970).

It is likely that B-chromosomes in Scilla autumnalis arise frequently since all Bs in the two tetraploid populations and three diploid populations known so far (Battaglia, 1963, 1964; Ruiz Rejón, 1980) are morphologically different. This suggests an independent origin.

Battaglia (1963, 1964) suggests that Bs in Scilla autumnalis could be derived by breakage of the nucleolar-organiser chromosome B3 across the N.O. region. However, he presents no evidence to substantiate this claim and in this thesis B-like novel chromosomes have been produced by deletion of several chromosome groups (Plate 5.1 a-aa).

# CHAPTER FIVE

## STRUCTURAL VARIATION IN SCILLA AUTUMNALIS

### I. Introduction

Mutation can be divided into two main classes: (i) gene or point mutation involving changes at the level of individual nucleotide pairs in the DNA helix and (ii) chromosomal mutation involving microscopically-visible chromosome segments which may contain several genetic loci. This latter class of chromosomal variation, which involves change in the linear arrangement of chromosomes, was largely ignored by the great geneticists of the past (Fisher, Haldane and Muller) and it was discounted as a major force in evolution until fairly recently (Darlington, 1956). This distinction has a functional basis, however, since chromosomal, unlike most genic, mutations may modify the course of meiosis. It is this type of structural variation which will be investigated in relation to Scilla autumnalis.

There are four main types of major chromosomal rearrangements which occur as a result of breakage with or without subsequent reunion.

1. Terminal deletions are the simplest type of structural change and require only one break with no subsequent reunion. Interstitial deletions require a minimum of two breaks. In both cases, however, an acentric fragment is produced which will subsequently be lost. Breaks through the centromere are the exception and both fission products may persist as telocentrics. Deletions involve loss of genetic material and are usually lethal in diploids though not in polyploids.

2. Interchange results from reciprocal exchange of terminal segments between two different chromosomes. This requires two breaks and two reunions. Interchanges can be symmetrical, if the exchanged segments

are of equal length or asymmetrical if the exchanged segments are of differing length. In the absence of suitable markers, such as C- and G-bands or nucleolar-organiser regions, symmetrical interchanges can only be detected during meiosis.

3. Inversions require two breaks in a single chromosome followed by reunion after a $180^{o}$ rotation. The inverted segment may (pericentric) or may not (paracentric) involve the centromere. Pericentric inversions may be detectable in mitosis if they are asymmetric with respect to the centromere leading to an apparent shift in centromere position (centric shift).

4. Duplications are readily detectable at mitosis due to a length difference in a pair of homologues. The duplicated segment may be terminal or interstitial and meiotic analysis is required to localise them. The origins of duplications are not well understood but errors of crossing-over may be involved as in the Bar locus of Drosophila melanogaster (Sturtevant, 1925).

Other structural rearrangements may occur involving more than two breaks but these are correspondingly less frequent in populations.

Structural rearrangements such as inversions and interchanges in the heterozygous condition, although genically balanced, may give rise to unbalanced gene combination at meiosis, some of which will be inviable. In the homozygous condition however meiotic behaviour is undistrubed.

Structural chromosome changes in natural populations are examples of discontinuous variants (Mather, 1953; 1973). Such variation may be present simply as a result of mutation or it may reach polymorphic proportions. Polymorphism has been defined as the occurrence together in the same interbreeding population of two or more forms of the same

species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation (Ford, 1940). Thus a functional definition of a polymorphism is used in which the rarest variant must exceed 5% (or, in some investigations, 2%) of the population. Now, since structural rearrangements such as inversions require two breaks it is likely that an independent origin of identical rearrangements is a very rare phenomenon, comparable in frequency to point mutation rate. We can therefore determine whether structural changes simply result from recurrent mutation or reach polymorphic proportions using a frequency of 5% as our discriminatory level.

Basic information on the cytological structure of populations is available for very few organisms other than Homo sapiens (Hook and Porter, 1977). The nature and extent of unique structural variation in diploid (BB), autotetraploid (BBBB) and autoallohexaploid (AABBBB) populations of Scilla autumnalis is considered in this chapter. Polymorphic variants will be discussed in Chapter 6.

## Results

### I. Intra-individual structural variation

As with numerical variation, structural changes may be essentially somatic affecting one or a few cells in a root, or may be characteristic of the whole plant. We will deal initially with somatic events.

#### a) Deletion

When spontaneous breakage occurs at a position other than the centromere an acentric fragment is produced. The nature of the fragment will depend on whether the break occurs in the G1 or G2 phase of the cell cycle. G1 breaks give rise to chromosome fragments and G2 breaks to chromatid fragments (Plate 5.1). Acentric fragments, lacking mobility, will be

Plate 5.1 Spontaneous cellular deletions

a-b Deletions in diploids

a 2 cells with a B5 deletion (long arm lost)
b B2 break (long arm) and B3 break (through NOR)

c-s Deletions in tetraploids

c B1 short arm break
d B5 break (through centromere) plus minute
e B5 chromatid break
f B3 break (through NOR)
g B5 long arm break
h chromatid fragment
i chromosome fragment
j B5 break plus minute
k 2 large and 2 small fragments (no obvious deletion)
l B2 long arm deletion
m B3 deletion plus two fragments
n B3 short arm deletion
o B3 deletion (long arm lost)
p B4 deletion (short arm lost)
q 2 cells; 1 with B7 deletion, 1 normal
r B7 deletion (both arms shorter)
s B7 deletion (one arm lost) plus minutes

t-aa Deletions in hexaploids

t B2 and B3 deletions plus 2 fragments
u B3 long arm break
v chromosome fragment
w chromosome fragment
x six fragments
y A1 deletion (long arm lost)
z A5 deletion (one arm lost)
aa B7 deletion (one arm lost)

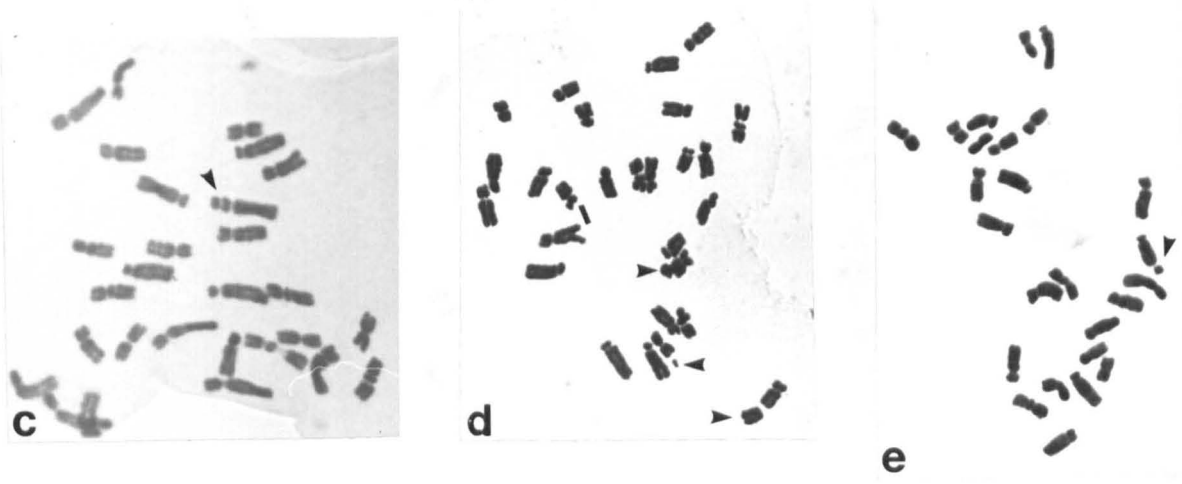
bb-ee Extreme fragmentation

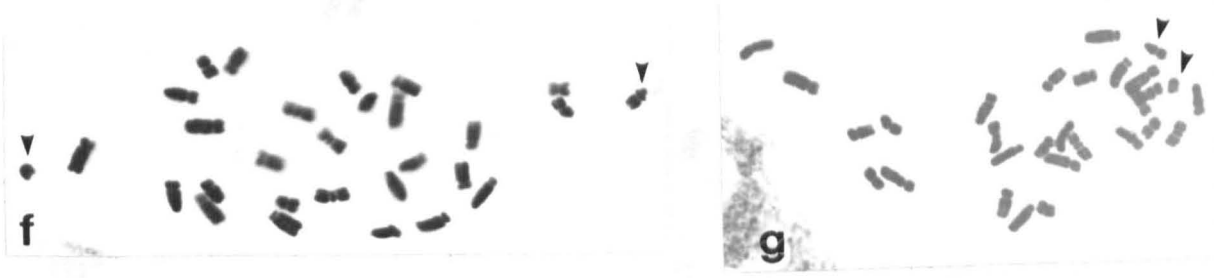
bb hexaploid root tip mitosis
cc tetraploid root tip mitosis
dd hexaploid meiosis: division I
ee hexaploid meiosis: division II

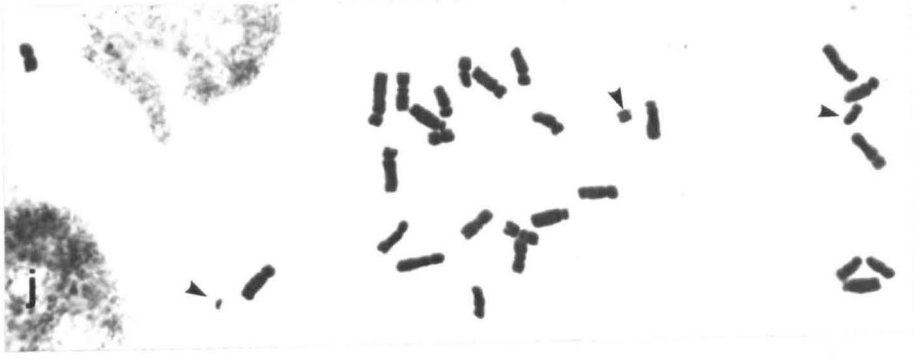
Plate 5.1

a-b Deletions in diploids


c-s Deletions in tetraploids

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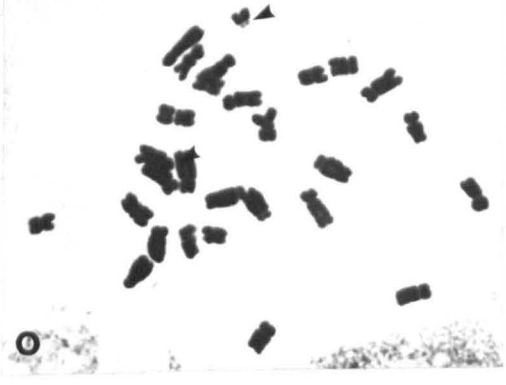
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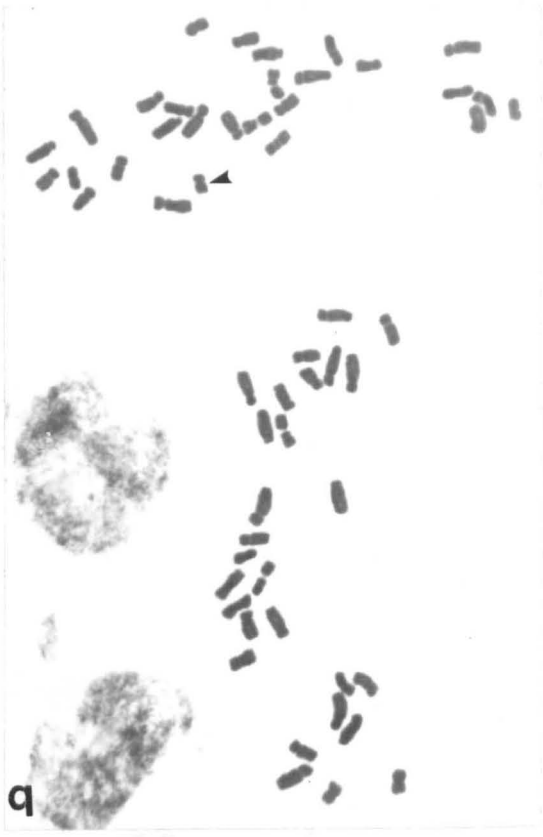


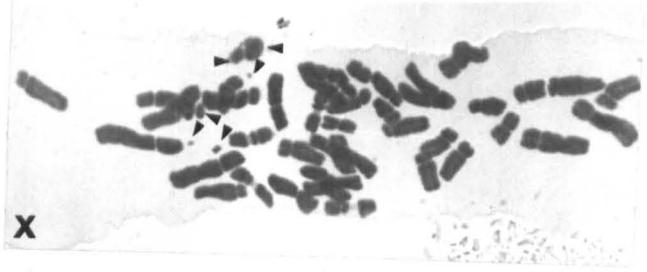



t-aa Deletions in hexaploids


u


v


w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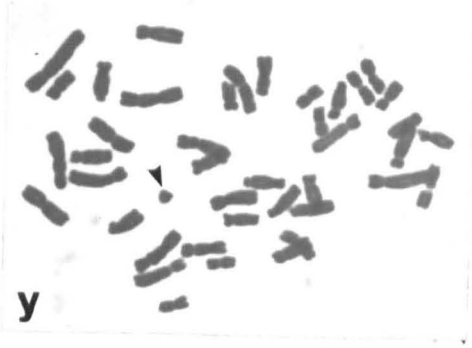
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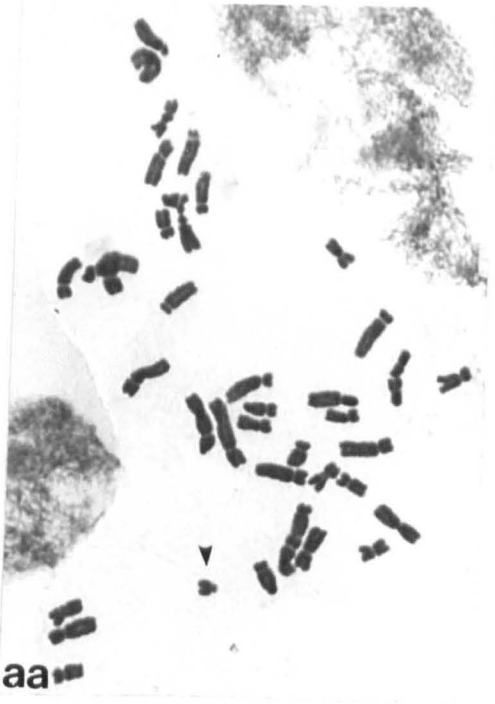
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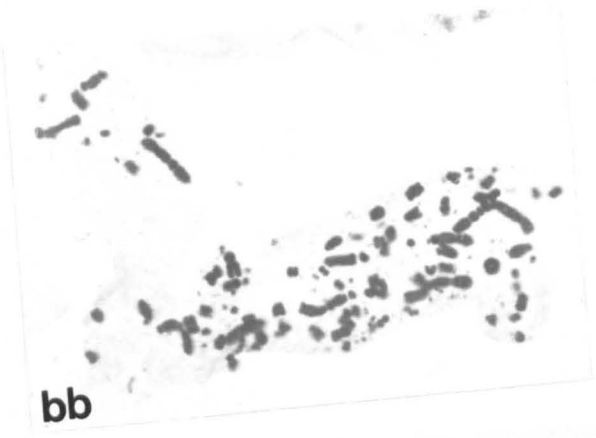
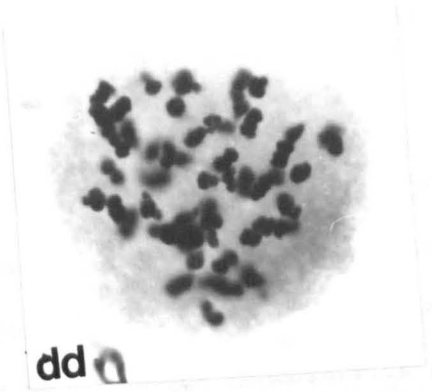
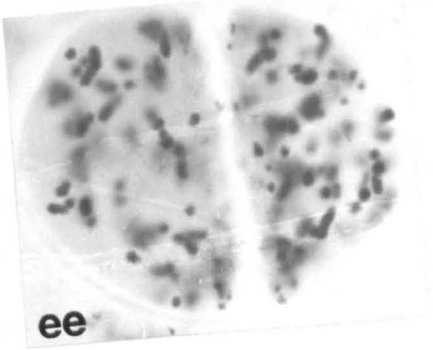

bb


cc


dd


ee

rapidly lost in a few cell generations and when observed, then, indicate a relatively recent breakage event. In Scilla autumnalis approximately equal numbers of chromatid and chromosome fragments have been observed in both tetraploids and hexaploids (Table 5.1). Mean fragment size did not vary greatly between chromatid and chromosome fragments or ploidy level (Table 5.1).

Up to six fragments per cell have been found (Plate 5.1x) although the majority contained only a single fragment (Table 5.2; Plate 5.1 ). The frequency of deletion cells which included fragments increases with ploidy level, from a diploid level of 0.45% to 1.7% in hexaploids, approximately a three-fold increase (Table 5.2). The frequency of all deletion cells with and without fragments similarly increases with ploidy level from 0.45% in diploids to 4.33% in hexaploids, a ten-fold increase.

A small number of cells from both hexaploid and tetraploid plants exhibited complete fragmentation of the complement (Plate 5.1 bb-ee). In these cells enormous numbers of very small fragments are seen. In addition, multicentric chromosomes and triradials occur at a low frequency in these cells. A catastrophic collapse of the DNA repair mechanism is indicated in cells containing these pulverised chromosomes.

b) Interchange

Spontaneous interchanges were observed in cells of tetraploid and hexaploid plants with a frequency of 0.25% and 0.32% respectively (Table 5.3). As a consequence of interchange a dicentric chromosome plus a fragment and two monocentrics should be produced with equal frequency.

Table 5.1 Chromatid and chromosome fragment size in cells of autotetraploids and autoallohexaploids at colchicine metaphase

| | 4x | | 6x | |
|---|---|---|---|---|
| | chromatid | chromosome | chromatid | chromosome |
| number | 17 | 11 | 6 | 6 |
| mean size (μm) | 1.13 | 1.63 | 1.17 | 2.30 |
| s.d. | 0.49 | 0.95 | 1.00 | 1.44 |

Table 5.2 The incidence of cells with varying numbers of fragments in root tips of diploid, autotetraploid and autoallohexaploid S. autumnalis. Cells were scored at colchicine - metaphase

| Number of fragments per cell | Number of cells | | |
|---|---|---|---|
| | 2x | 4x | 6x |
| 0 | 664 (99.4%) | 11,088 (99.3%) | 2,499 (98.3%) |
| 1 | 3 (0.45%) | 71 (0.6%) | 33 (1.3%) |
| 2 | | 5 | 7 |
| 3 | | 2 | 3 |
| 4 | | 1 0.1% | - 0.4% |
| 5 | | 2 | 1 |
| 6 | | - | 1 |
| Total cells | 668 | 11,169 | 2,543 |

Table 5.3 Summary of the incidence of spontaneous deletion, interchange and inversion in colchicine metaphase root tip cells of diploid, autotetraploid and auto-allohexaploid plants of S. autumnalis

| Ploidy level | Cells scored | Deletions | Interchanges | Inversions | Total | Total per genome |
|---|---|---|---|---|---|---|
| 2x | 668 | 3 (0.45%) | - | - | 3 (0.45%) | 0.45% |
| 4x | 11,169 | 186 (1.67%) | 21 (0.25%) | 1 (0.01%) | 208 (1.86%) | 0.93% |
| 6x | 2,543 | 110 (4.33%) | 8 (0.32%) | 2 (0.08%) | 120 (4.72%) | 1.57% |
| Total cells | | 299 (2.08%) | 29 (0.20%) | 3 (0.02%) | 331 (2.30%) | - |

Though both types of product were observed (Plate 5.2 ), monocentric interchange products were more common than dicentric products (20:9), attributable to the mitotic inefficiency of dicentrics. Elimination of dicentrics with long intercentric distances is likely to be rapid. In Hypochoeris maculata seedlings, for example, selection against abnormal complements with multicentric chromosomes is rapid, operating over a three-day time span (Parker, 1971).

An obviously complex situation has been found in the hexaploid plant LL4, which involved cells with one or two dicentrics (Plate 5.2 i,j).

c) Inversion

Spontaneous inversions were extremely rare with only three in a total of 14,280 cells, an overall frequency of 0.021% (Table 5.3; Plate 5.3 a,b).

Mosaic plants

Four tetraploid plants were chromosomally mosaic in root tip cells.

(i) GP27

In GP27 about 10% of cells contained a B2 chromosome with a long arm deletion (Fig. 5.1). 5.5 μm (90%) of the long arm was deleted in the variant chromosome.

(ii) PH1

The situation in PH1 was more complex. Five of ten cells in the two roots examined carried a B3 in which the long arm segment distal to the NOR was absent. Four of the other five cells had a standard karyotype while the fifth contained a small fragment.

The five cells carrying the B3 deletion were themselves of three distinct karyotypes due to further structural changes:

a) 2 cells with a B4 long arm deletion (51%).

Plate 5.2 Spontaneous cellular interchanges

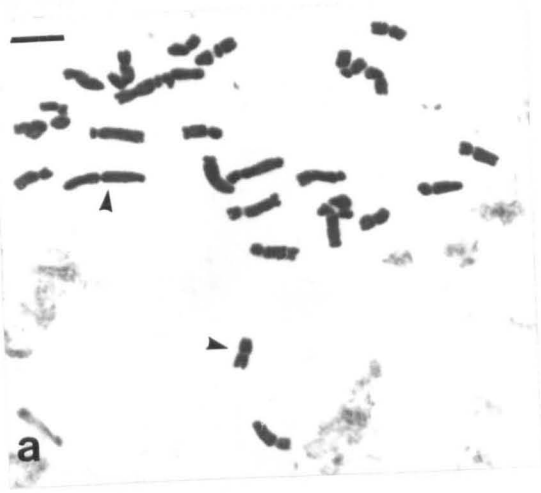
a-g Interchanges in tetraploids

a B1-B4 whole arm exchange
b B1-B5 whole arm exchange
c B1/B6 dicentric
d B4 dicentric (no reciprocal loss)
e B2/B3 dicentric plus fragment
f B1/B4 dicentric plus centric fragment
g B2/B4 dicentric

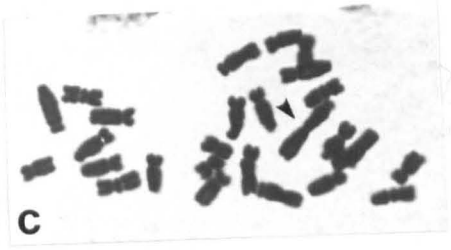
h-j Interchanges in hexaploids

h Dicentric
i Dicentric (LL4)
j 2 dicentrics (LL4)

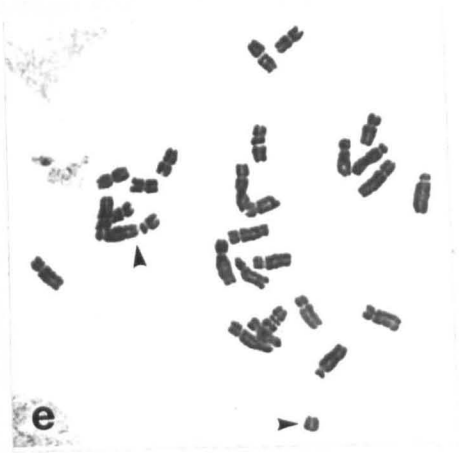
a-g tetraploids

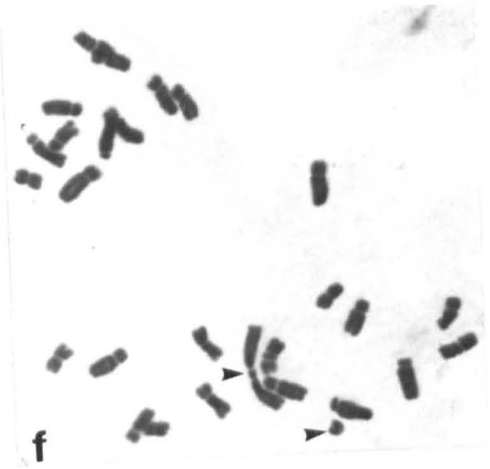




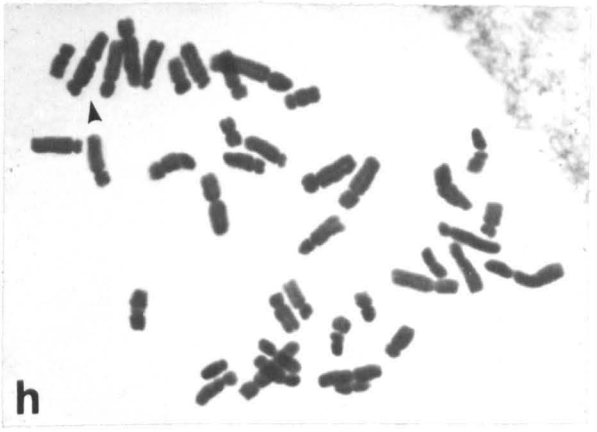







h-j tetraploids





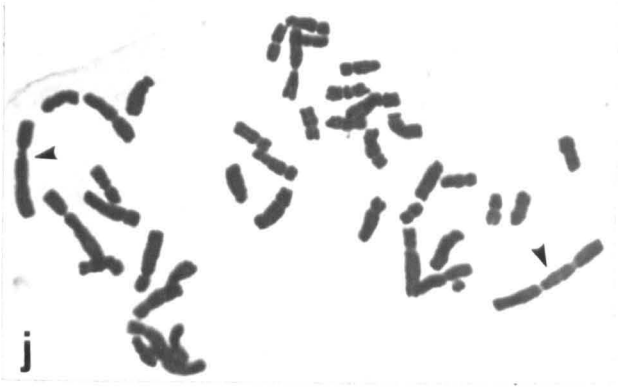
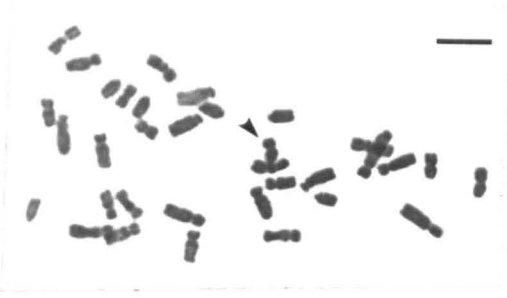
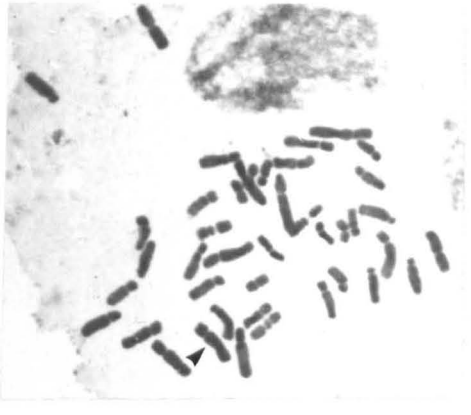


Plate 5.3 Spontaneous cellular inversions



(a) B7 inversion in a tetraploid (2n = 28 + 6B)



(b) A5 inversion in a hexaploid (2n = 42)

Figures 5.1 - 5.3 Chromosomal variants of *Scilla autumnalis*

Figure 5.1 Deletions

Figure 5.2 Inversions

Figure 5.3 Duplications

Unique variants are given their population number (e.g. CCF28). Polymorphic variants are given the polymorphism number (e.g. Inv 3-1).

Figure 5.1 Deletions

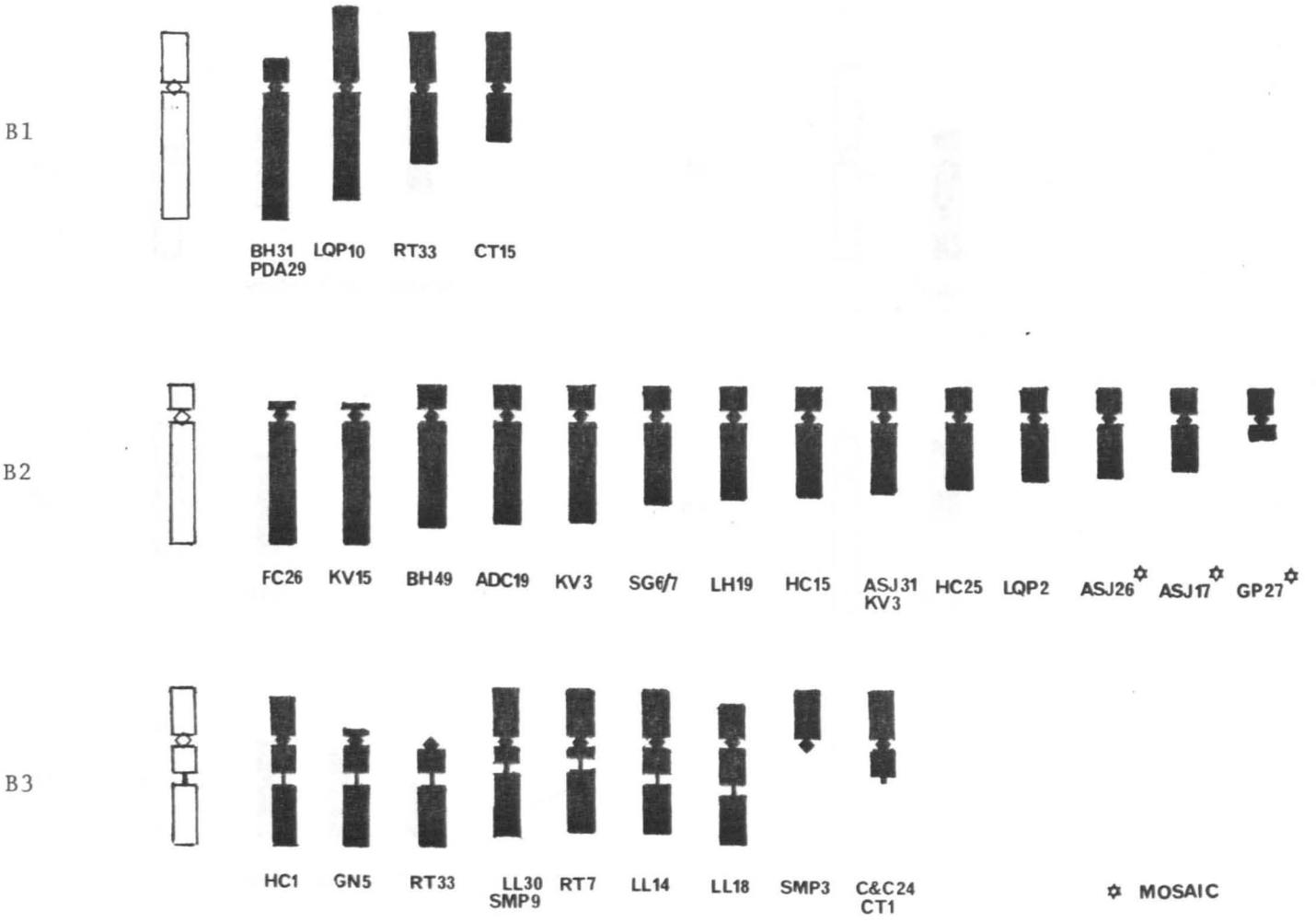


Figure 5.1 continued

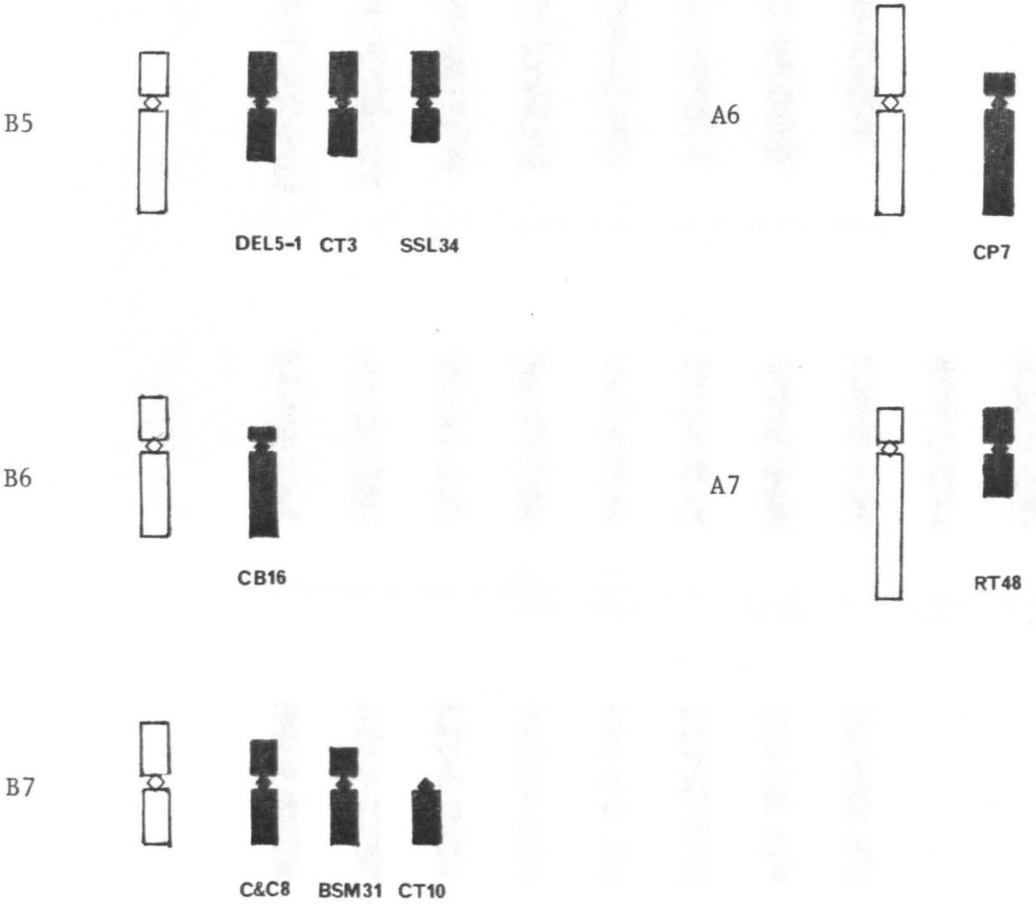


Figure 5.2 Inversions

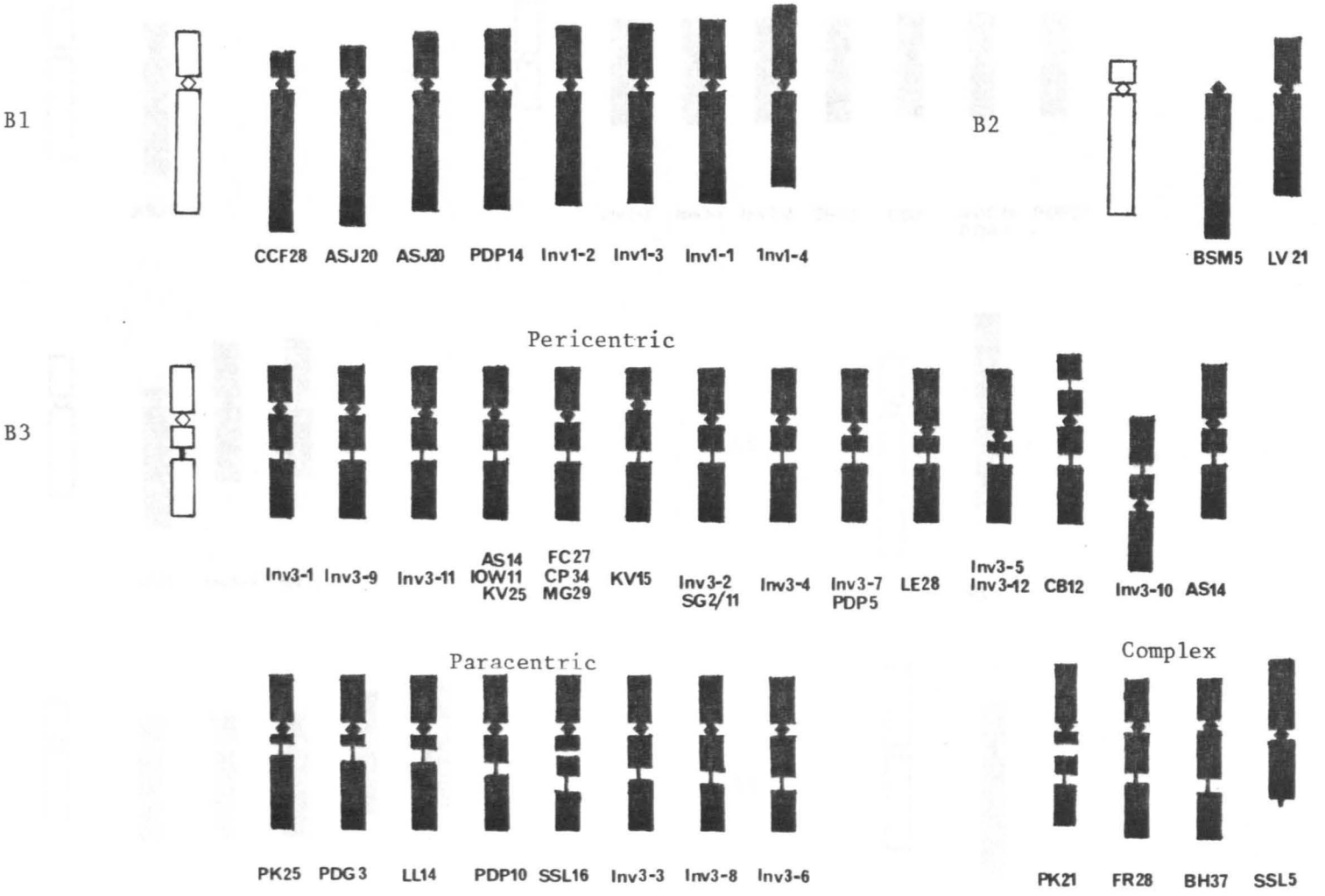


Figure 5.2 continued

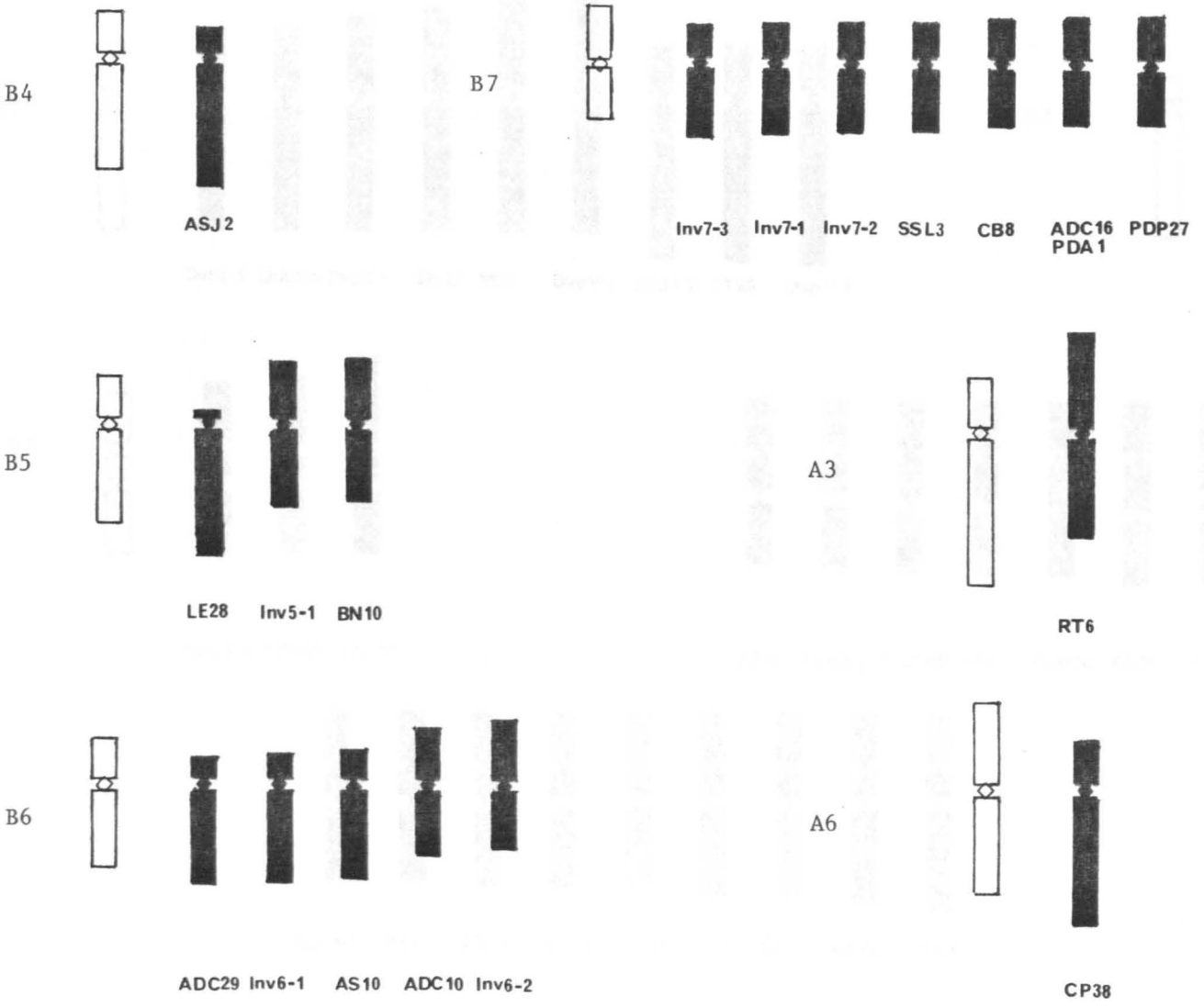


Figure 5.3 Duplications

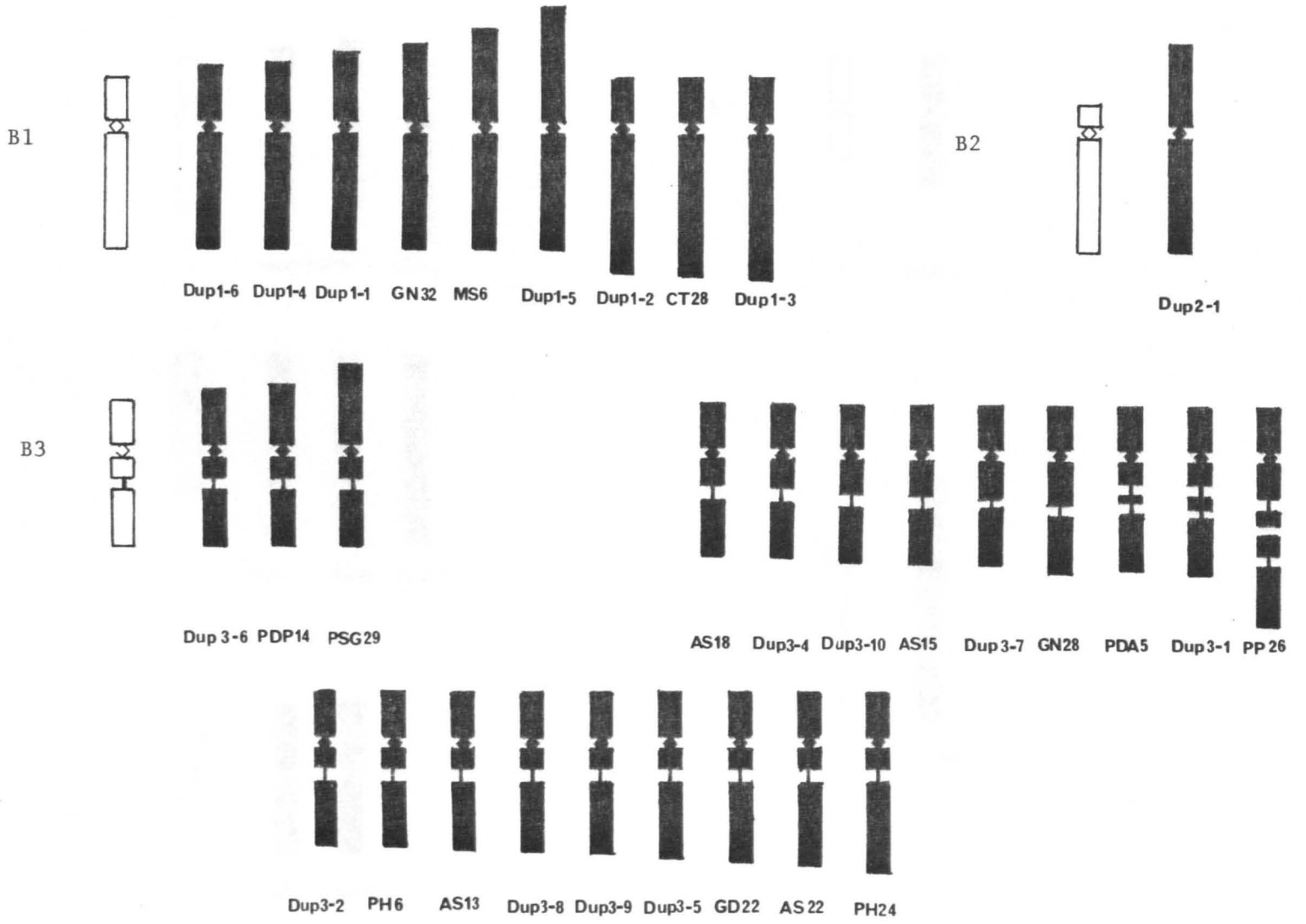


Figure 5.3 continued

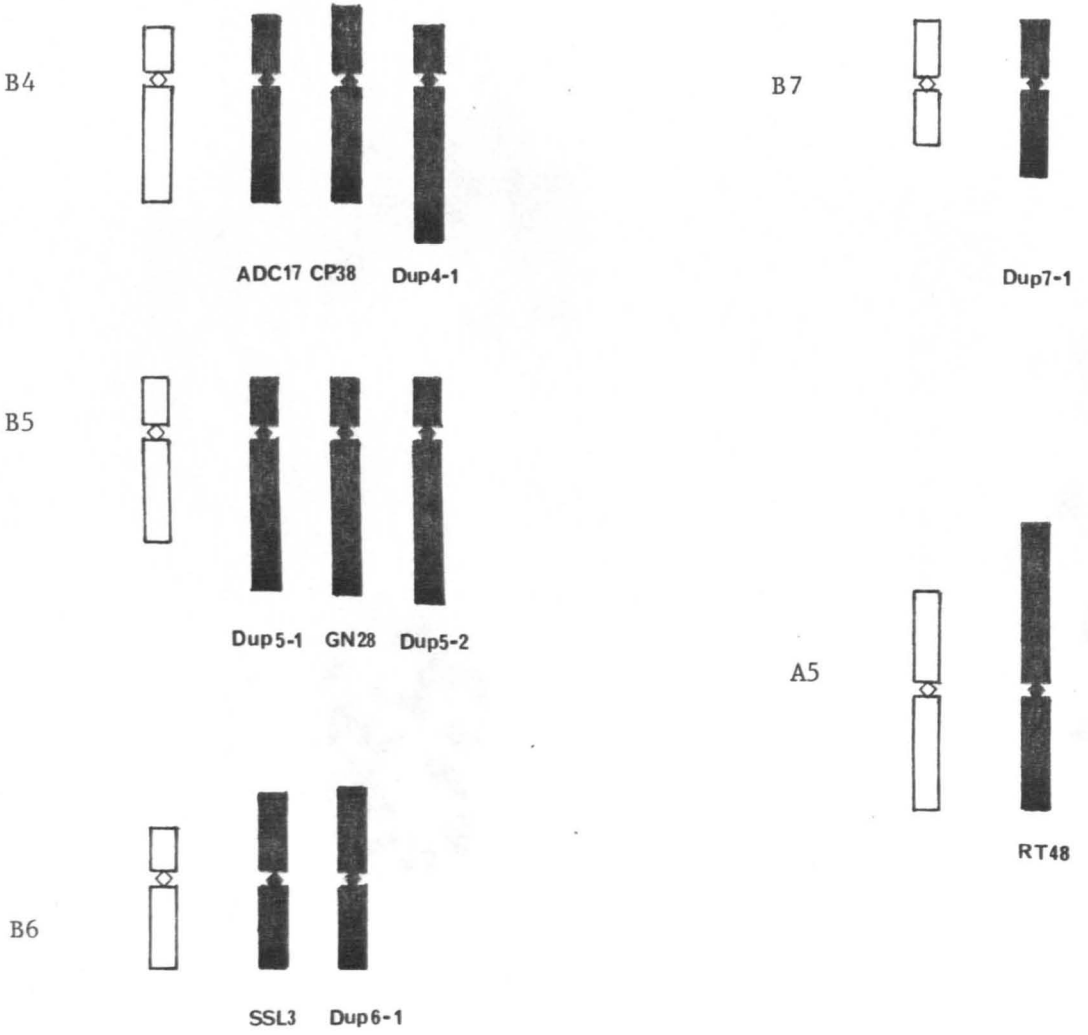
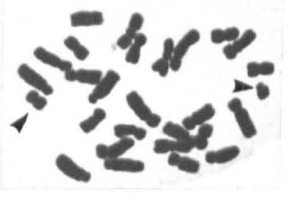


Plate 5.4 Chromosomally mosaic plants

(a) ASJ26 B2 deletion

(b) PH1 B3 deletion



(c) B3 and B5 deletions

b) 2 cells with a B5 long arm deletion (77%).

c) 1 cell with a distinct B5 long arm deletion (55%) and an interchange between B2 long arm and B7.

Interphase cells in both roots frequently contained micronuclei indicating a general mitotic instability of the complement (Plate 5.9).

(iii) ASJ17

This plant was mosaic for a deletion in one B2 chromosome which reduced the length of the long arm by 3.8 μm (63.5%) (Table 5.4; Fig. 5.1).

(iv) ASJ26

ASJ26 was mosaic for a B2 deletion which reduced the length of the long arm by 3.4 μm (56%) (Table 5.5; Fig. 5.1; Plate 5.4a). This plant was also simplex for a polymorphic B3 inversion (Inv 3-13).

Comparison of structural changes

The overall level of spontaneous interchange was only marginally greater for hexaploids than for tetraploids: 0.32% of cells as opposed to 0.25% (Table 5.3). The frequency of spontaneous interchanges and inversions was, however, lower than that of deletion at all three levels of ploidy. This is expected since an interchange requires two breaks and reunions whilst a deletion requires only a single break. The extremely low frequency of inversion may in part be due to problems of detection but is nevertheless surprising in view of the high frequency of pericentric inversions affecting whole plants (see Chapter 6).

II. Inter-individual (whole plant) variation

Chromosomal variants, in which all root-tip cells were affected, were found at all levels of ploidy - diploid, autotetraploid and autoallohexaploid. Four main types of chromosomal rearrangement were distinguished:

Table 5.4 The numbers of spontaneous structural changes affecting different chromosome groups in colchicine metaphase root tip cells of diploid, autotetraploid and autoallohexaploid S. autumnalis. (Expected numbers calculated on the basis of chromosome length.)

| | A genome | | | | | | | | B genome | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | A4 | A5 | A6 | A7 | Total | B1 | B2 | B3 | B4 | B5 | B6 | B7 | Total | | $\chi^2$ | P |
| Deletions | | | | | | | | | | | | | | | | | | | |
| 2x | – | – | – | – | – | – | – | 0 | – | – | – | – | 1 | – | – | 1 | } | | |
| 4x | – | – | – | – | – | – | – | 0 | 7 | 12 | 15 | 9 | 5 | 5 | 8 | 61 | } | 75 | |
| 6x | 3 | – | 1 | – | – | 2 | 2 | 8 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 12 | } | | |
| Total obs. | | | | | | | | | 9 | 14 | 17 | 10 | 7 | 7 | 10 | | | | |
| exp. | | | | | | | | | 13.6 | 11.5 | 10.3 | 11.4 | 10.4 | 9.1 | 7.8 | | | 8.84 | > 0.2 |
| Interchanges | | | | | | | | | | | | | | | | | | | |
| 4x | – | – | – | – | – | – | – | 0 | 8 | 6 | 4 | 6 | 4 | 4 | 4 | 36 | } | | |
| 6x | 1 | – | – | 1* | 1 | – | – | 3 | – | 1* | – | – | – | – | – | 1 | } | 37 | |
| Total exp. | | | | | | | | | 6.6 | 6.0 | 5.0 | 5.5 | 5.0 | 4.4 | 3.8 | | | 0.76 | > 0.9 |
| Inversions | | | | | | | | | | | | | | | | | | | |
| 4x | – | – | – | – | – | – | – | 0 | – | – | – | – | – | 1 | – | 1 | } | | |
| 6x | – | – | – | – | – | 1 | – | 1 | – | – | – | – | 1 | – | – | 1 | } | 2 | |
| Total changes obs. | 4 | 0 | 1 | 1 | 1 | 3 | 2 | 12 | 17 | 20 | 21 | 16 | 12 | 12 | 14 | 112 | | | |
| exp. | | | | | | | | | 20.5 | 17.5 | 15.6 | 17.2 | 15.7 | 13.8 | 11.8 | | | 4.48 | > 0.25 |

* A/B interchange

Table 5.5 Unique structural variation affecting whole plants of Scilla autumnalis. The chromosome arm affected is shown (s = short arm; l = long arm). Inversions are pericentric (peri), paracentric (para) or complex. Complex inversions affecting the N.O. chromosome (B3) change the lengths of all three chromosome segments.

| Plant | Type of Change | Chromosome and arm affected | Change in length (μm) | Arm ratio | Pollen stainability (%) |
|---|---|---|---|---|---|
| 2x | | | | | |
| PK25 | Inv. | B3 (para) | - | 1:1.75 | - |
| PK21 | Inv. | B3 (complex) | - | 1:1.2 | - |
| PK21 | Centric fission | B6 - | - | - | |
| MS6 | Dup. | B1 s | 2.5 | 1:1.25 | - |
| 4x | | | | | |
| BN10 | Inv. | B5 (peri) | - | 1:1.15 | 99 |
| PH1 | Del.* | B3 l | 3.0 | 1:2.1 | - |
| PH6 | Dup. | B3 l | 0.5 | 1:1.9 | - |
| PH24 | Dup. | B3 l | 1.8 | 1:2.48 | - |
| C+C8 | Del. | B7 - | 0.8 | 1:1.5 | 98 |
| C+C24 | Del. | B3 l | 3.0 | 1:2.1 | - |
| GP7 | Int. | B2 l | - | 1:2.08 | 99 |
| | | B7 - | - | 1:2.39 | - |
| GP27 | Del.* | B2 l | 5.5 | 1:2.0 | - |
| PP26 | Dup. | B3 l | 3.0 | 1:3.0 | 85 |
| BH31 | Del. | B1 s | 2.2 | 1:5.1 | - |
| BH37 | Inv. | B3 (complex) | - | 1:2.71 | - |
| BH49 | Del. | B2 l | 0.9 | 1:4.33 | 100 |
| LQP2 | Del. | B2 l | 3.4 | 1:2.29 | 89 |
| LQP10 | Del. | B1 l | 0.9 | 1:1.44 | - |
| IOW11 | Inv. | B3 (peri) | - | 1:2.28 | - |
| HC1 | Del. | B3 s | 0.5 | 1:2.51 | - |
| HC15 | Del. | B2 l | 2.5 | 1:3.0 | - |
| HC21 | Int. | $B4^{l}$ $B6^{s}$ | - | - | - |
| HC25 | Del. | B2 l | 2.9 | 1:2.42 | - |
| GN5 | Del. | B3 s | 2.1 | 1:13.8 | 100 |
| GN28 | Dup. | B3 l | 1.1 | 1:2.21 | 92 |
| GN28 | Dup. | B5 l | 2.4 | 1:3.41 | - |
| GN32 | Dup. | B1 s | 1.7 | 1:1.51 | 96 |
| CB8 | Inv. | B7 (peri) | - | 1:1.43 | - |
| CB12 | Inv. | B3 (peri) | - | 1:1.87 | - |
| CB16 | Del. | B6 s | 1.3 | 1:5.85 | - |
| FR16 | Int. | B1 l | 1.5 | 1:3.18 | - |
| | | B6 l | 1.5 | 1:1.9 | - |
| FR28 | Inv. | B3 (complex) | - | 1:2.33 | - |
| FC26 | Del. | B2 s | 0.7 | 1:12.34 | - |
| FC27 | Inv. | B3 (peri) | - | 1:2.17 | - |
| CCF28 | Inv. | B1 (peri) | - | 1:5.44 | - |

/cont.....

Table 5.5 continued

| Plant | Type of change | Chromosome and arm affected | Change in length (µm) | Arm ratio | Pollen stainability (%) |
|---|---|---|---|---|---|
| SG2/11 | Inv. | B3 (peri) | - | 1:2.04 | - |
| SG6/7 | Del. | B2 l | 2.1 | 1:3.38 | - |
| MG29 | Inv. | B3 (peri) | - | 1:2.1 | 0 |
| LH19 | Del. | B2 l | 2.4 | 1:3.1 | - |
| BSM5 | Inv. | B2 (peri) | - | - | - |
| BSM31 | Del. | B7 - | 1.2 | 1:2.0 | - |
| LV28 | Inv. | B2 (peri) | - | 1:2.2 | - |
| PDG3 | Inv. | B3 (para) | - | 1:1.73 | - |
| PDP5 | Inv. | B3 (peri) | - | 1:1.43 | - |
| PDP10 | Inv. | B3 (para) | - | 1:1.73 | - |
| PDP14 | Inv. | B1 (peri) | - | 1:2.35 | - |
| PDP14 | Dup. | B3 s | 0.9 | 1:1.28 | - |
| PDP27 | Inv. | B7 - | - | 1:1.25 | - |
| PSG29 | Dup. | B3 s | 1.9 | 1:1.04 | 61 |
| SSL3 | Dup. | B6 s | 1.5 | 1:1.06 | - |
| SSL3 | Inv. | B7 (peri) | - | 1:1.56 | 89 |
| SSL5 | Inv. | B3 (complex) | - | 1:1.23 | - |
| SSL16 | Inv. | B3 (para) | - | 1:1.73 | 71 |
| SSL34 | Del. | B5 l | 3.2 | 1:1.46 | - |
| ADC10 | Inv. | B6 (peri) | - | 1:1.25 | - |
| ADC16 | Inv. | B7 (peri) | - | 1:1.30 | - |
| ADC17 | Dup. | B4 s | 0.7 | 1:1.98 | - |
| ADC19 | Del. | B2 l | 1.0 | 1:4.25 | - |
| ADC29 | Inv. | B6 (peri) | - | 1:4.18 | - |
| ASJ2 | Inv. | B4 (peri) | - | 1:5.08 | 74 |
| ASJ17 | Del.* | B2 l | 3.8 | 1:1.88 | - |
| ASJ20 | Inv. | B1 (peri) | - | 1:4.12 | - |
| ASJ20 | Inv. | B1 (peri) | - | 1:2.41 | - |
| ASJ26 | Del.* | B2 l | 3.4 | 1:2.25 | - |
| ASJ31 | Del. | B2 l | 2.6 | 1:2.92 | - |
| PDA1 | Inv. | B7 (peri) | - | 1:1.26 | - |
| PDA5 | Dup. | B3 l | 0.4 | 1:1.96 | 94 |
| PDA29 | Del. | B1 s | 2.2 | 1:5.21 | - |
| AS10 | Inv. | B6 (peri) | - | 1:3.33 | - |
| AS13 | Dup. | B3 l | 0.6 | 1:1.98 | - |
| AS14 | Inv. | B3 (peri) | - | 1:2.28 | - |
| AS14 | Inv. | B3 (peri) | - | 1:1.42 | - |
| AS15 | Dup. | B3 l | 0.8 | 1:2.06 | - |
| AS18 | Dup. | B3 l | 0.4 | 1:1.90 | - |
| AS22 | Dup. | B3 l | 1.5 | 1:2.34 | - |
| KV3 | Del. | B2 l | 1.1 | 1:4.17 | - |
| KV3 | Del. | B2 l | 2.6 | 1:2.92 | - |
| KV15 | Inv. | B3 (peri) | - | 1:2.11 | - |
| KV15 | Del. | B2 s | 0.8 | 1:17.57 | - |
| KV25 | Inv. | B3 (peri) | - | 1:2.28 | - |

/cont.....

Table 5.5 continued

| Plant | Type of change | Chromosome and arm affected | Change in length (µm) | Arm ratio | Pollen stainability (%) |
|---|---|---|---|---|---|
| 6x | | | | | |
| CP7 | Del. | A6 s | 3.1 | 1:5.0 | - |
| CP34 | Inv. | B3 (peri) | - | 1:2.2 | 89 |
| CP38 | Inv. | A6 (peri) | - | 1:3.02 | - |
| CP38 | Dup. | B4 s | 0.6 | 1:1.78 | - |
| CT1 | Del. | B3 1 | 3.0 | 1:2.09 | 74 |
| CT3 | Del. | B5 1 | 2.5 | 1:1.0 | - |
| CT10 | Del. | B7 - | 2.5 | - | - |
| CT15 | Del. | B1 1 | 2.7 | 1:1.45 | - |
| CT28 | Dup. | B1 1 | 1.4 | 1:3.13 | - |
| GD6 | Del. | B7 - | - | - | 85 |
| GD22 | Dup. | B3 1 | 1.3 | 1:2.27 | 85 |
| RT6 | Inv. | A3 (peri) | - | 1:1.0 | - |
| RT7 | Del. | B3 1 | 0.5 | 1:1.31 | - |
| RT33 | Del. | B1 1 | 3.7 | 1:1.0 | - |
| RT33 | Del. | B3 s | 2.4 | - | - |
| RT48 | Dup. | A5 s | 3.2 | 1:1.39 | - |
| RT48 | Del. | A7 1 | 4.7 | 1:1.19 | 64 |
| LE28 | Inv. | B5 (peri) | - | 1:15.63 | - |
| SMP3 | Del. | B3 1 | 4.1 | - | - |
| SMP9 | Del. | B3 1 | 0.5 | 1:1.5 | - |
| LL14 | Del. | B3 1 | 0.6 | 1:1.46 | 94 |
| LL14 | Inv. | B3 (para) | - | 1:1.73 | - |
| LL18 | Del. | B3 1 | 0.8 | 1:2.14 | 99 |
| LL30 | Del. | B3 1 | 0.5 | 1:1.5 | 99 |

* mosaic

deletion, inversion, interchange and duplication. In the absence of meiotic evidence, centric shifts have been referred to as 'inversions' for the sake of convenience.

In this chapter unique variants - those which do not reach polymorphic proportions - will be considered. Polymorphic variation will be discussed in the following chapter. Information on pollen stainability is included for some of these unique variants and details of all variants are given in Table 5.5.

a) Diploids

Mt. Pantokrator

PK21

A B3 chromosome in this plant resulted from a complex inversion (Plate 5.6a; Fig. 5.2). This unique chromosome cannot be derived from a standard B3 chromosome by two breaks. An additional constriction is present between centromere and NOR. This plant also carried a B1 chromosome with a long arm duplication which reaches polymorphic proportions in the PK population. In addition, PK21 is a B6 fission heterozygote.

PK25

PK25 was heterozygous for a paracentric inversion in the long arm of B3, detectable due to a shift in the position of the nucleolar-organiser region to a more proximal position (Fig. 5.2; Plate 5.6b).

Meseena

MS6

This plant was heterozygous for a duplication of chromosome 1 which increased the length of the short arm by 2.5 μm (Fig. 5.3; Plate 5.7a).

Plate 5.5 Unique deletions

a-s Deletions in tetraploids

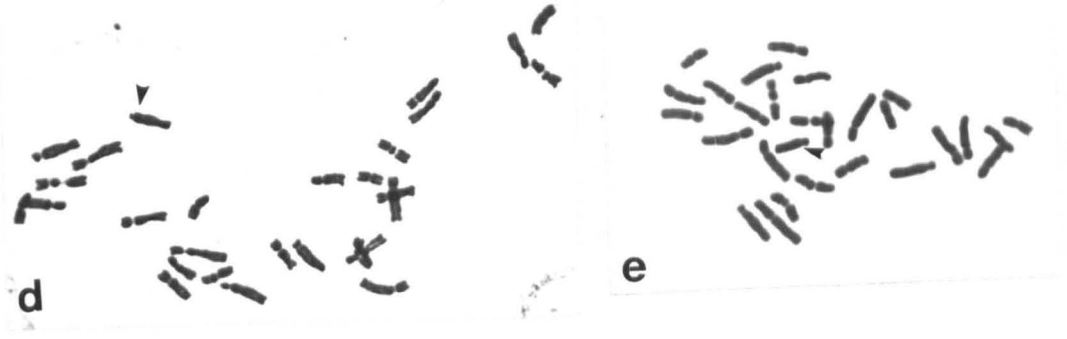
| | | |
|---|---|---|
| a | BH31 | B1 deletion |
| b | LQP10 | B1 deletion/duplication |
| c | PDA29 | B1 deletion |
| d | FC26 | B2 deletion |
| e | BH49 | B2 deletion |
| f | ADC19 | B2 deletion |
| g | KV3 | Two B2 deletions |
| h | SG6/7 | B2 deletion |
| i | HC15 | B2 deletion |
| j | LH19 | B2 deletion |
| k | HC25 | B2 deletion |
| l | LQP2 | B2 deletion |
| m | HC1 | B3 deletion |
| n | GN5 | B3 deletion |
| o | C+C24 | B3 deletion |
| p | SSL34 | B5 deletion |
| q | CB16 | B6 deletion |
| r | C+C8 | B7 deletion |
| s | BSM31 | B7 deletion |

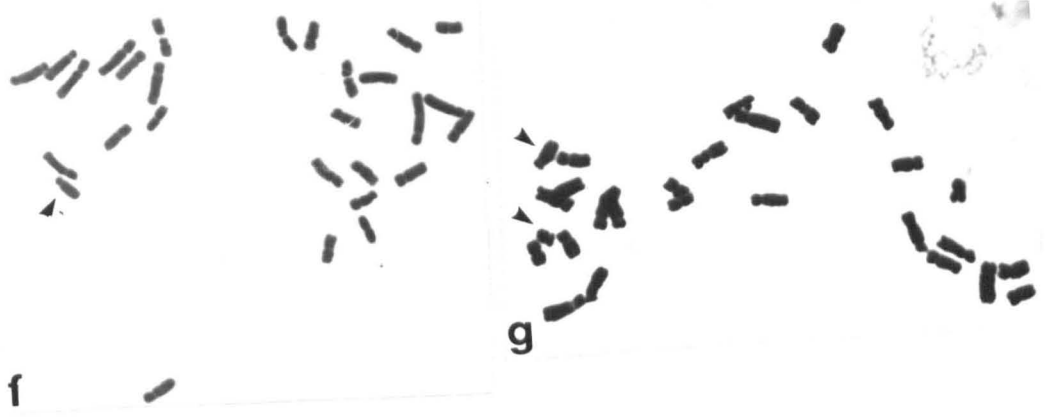
t-aa Deletions in hexaploids

| | | |
|---|---|---|
| t | RT33 | B1 and B3 deletions |
| u | LL30 | B3 deletion |
| v | SMP9 | B3 deletion |
| w | RT7 | B3 deletion |
| x | LL14 | B3 deletion and B3 paracentric inversion |
| y | LL18 | B3 deletion |
| z | CT10 | B7 deletion |
| aa | CP7 | A6 deletion |

a-s Tetraploids



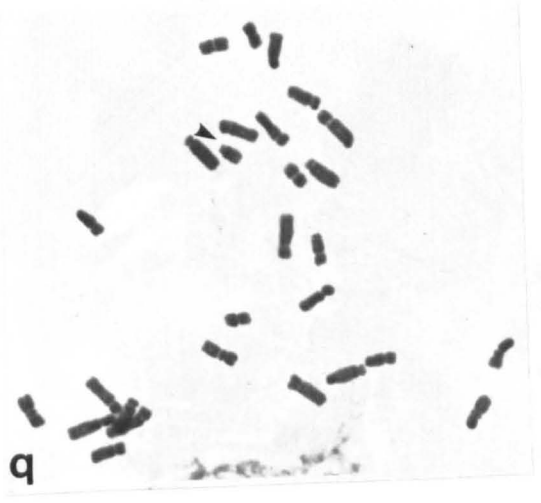


h
i
j
k
l
m
n


o

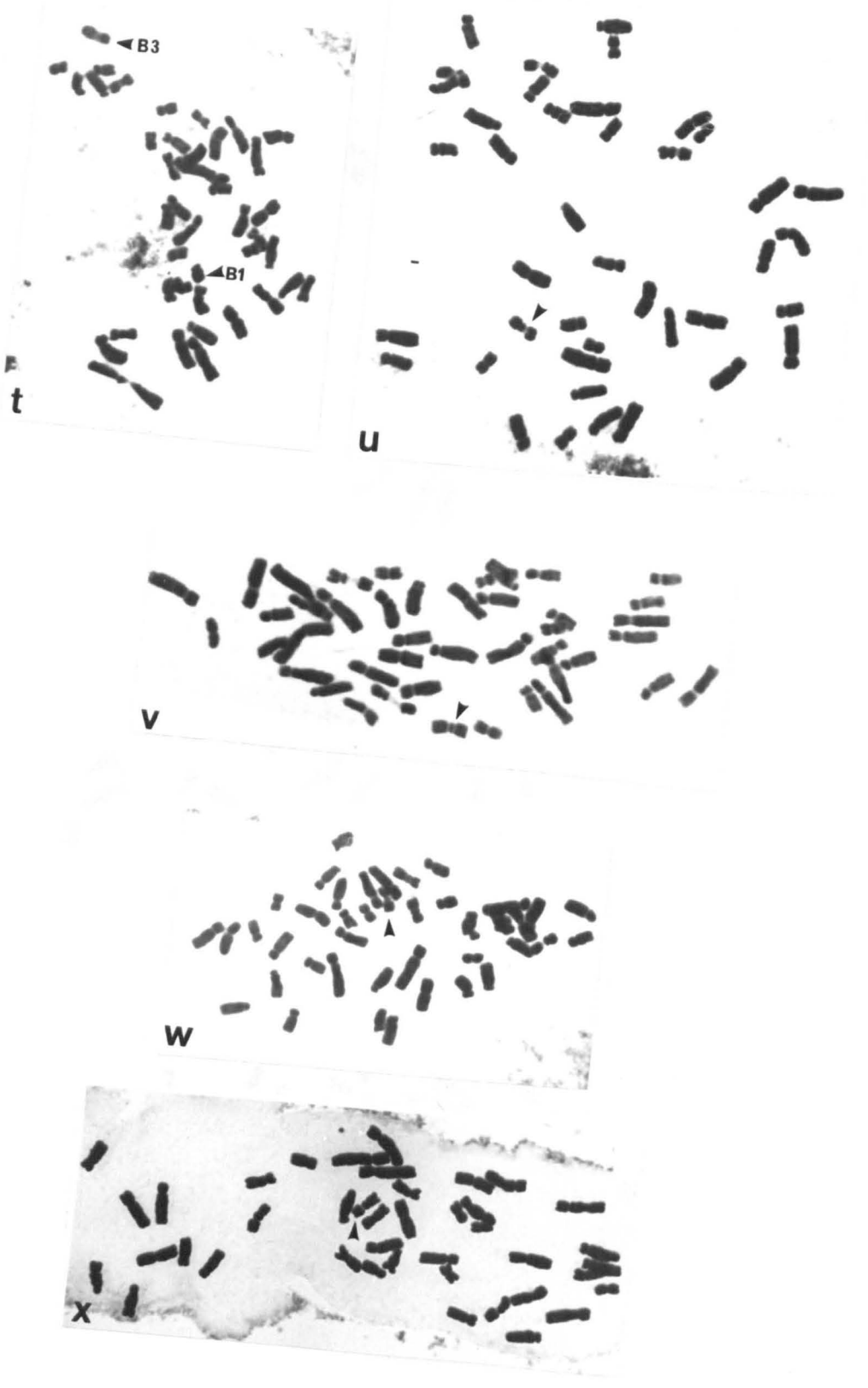

p

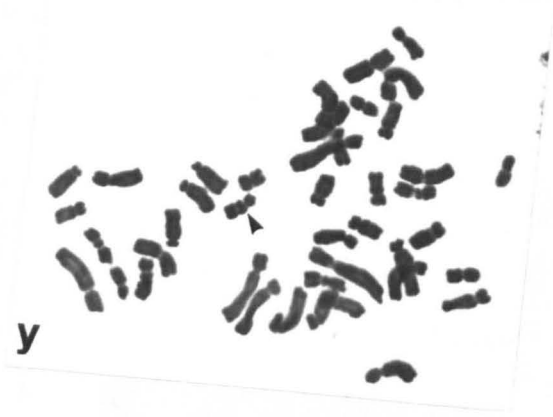

q


r


s

t-aa Hexaploids




y


z


aa

Plate 5.6 Unique inversions

a-b Inversions in diploids

| | | |
|---|---|---|
| a | PK21 | B3 complex inversion |
| b | PK25 | B3 paracentric inversion |

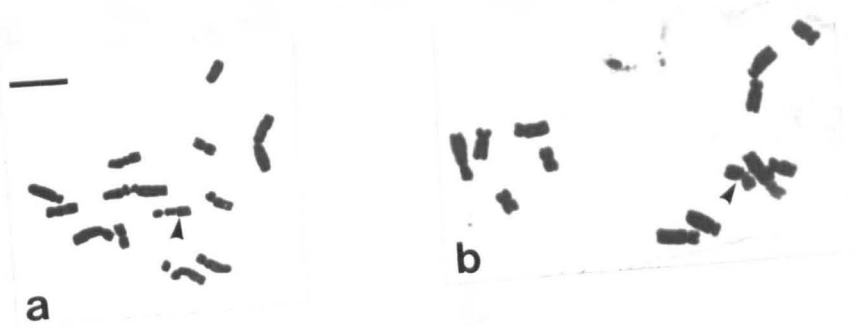
c-dd Inversions in tetraploids

| | | |
|---|---|---|
| c | CC28 | B1 pericentric inversion |
| d | ASJ20 | Two B1 pericentric inversions |
| e | PDP14 | B1 pericentric inversion |
| f | BSM5 | B2 pericentric inversion |
| g | LV28 | B2 pericentric inversion |
| h | AS14 | Two B3 pericentric inversions |
| i | IOW11 | B3 pericentric inversion |
| j | KV25 | B3 pericentric inversion |
| k | FC27 | B3 pericentric inversion |
| l | MG29 | B3 pericentric inversion |
| m | KV15 | B3 pericentric inversion |
| n | CB12 | B3 pericentric inversion |
| o | PDP5 | B3 pericentric inversion |
| p | PDG3 | B3 paracentric inversion |
| q | PDP10 | B3 paracentric inversion |
| r | SSL16 | B3 paracentric inversion |
| s | FR28 | B3 complex inversion |
| t | BH37 | B3 complex inversion |
| u | SSL5 | B3 complex inversion |
| v | ASJ2 | B4 pericentric inversion |
| w | BN10 | B5 pericentric inversion |
| x | ADC29 | B6 pericentric inversion |
| y | AS10 | B6 pericentric inversion |
| z | ADC10 | B6 pericentric inversion |
| aa | CB8 | B7 pericentric inversion |
| bb | ADC16 | B7 pericentric inversion |
| cc | PDA1 | B7 pericentric inversion |
| dd | PDP27 | B7 pericentric inversion |

ee-hh Inversions in hexaploids

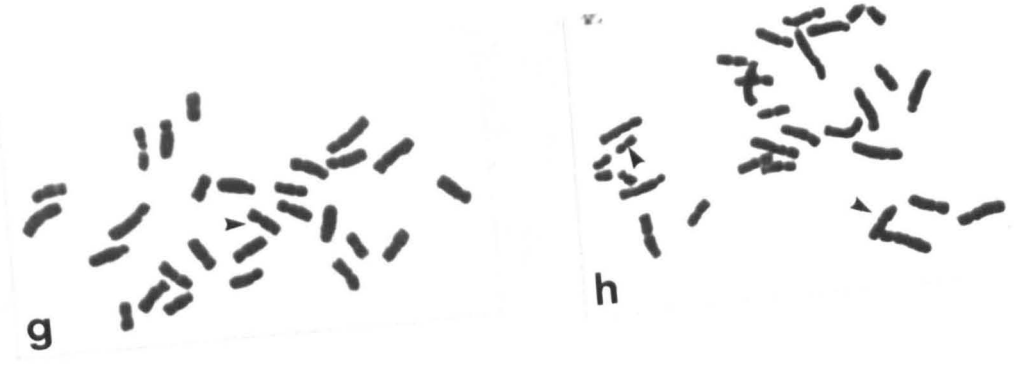
| | | |
|---|---|---|
| ee | CP34 | B3 pericentric inversion |
| ff | LE28 | B3 and B5 pericentric inversions |
| gg | RT6 | A3 pericentric inversion |
| hh | CP38 | A6 pericentric inversion |

a-b Diploids




c-dd Tetraploids

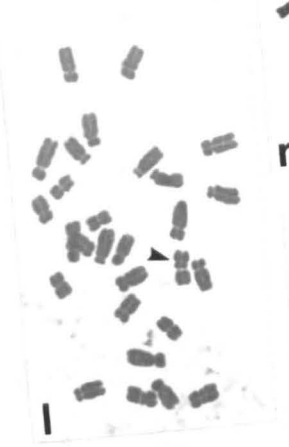





i


j


k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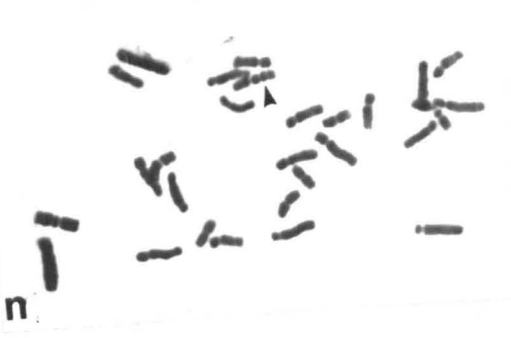
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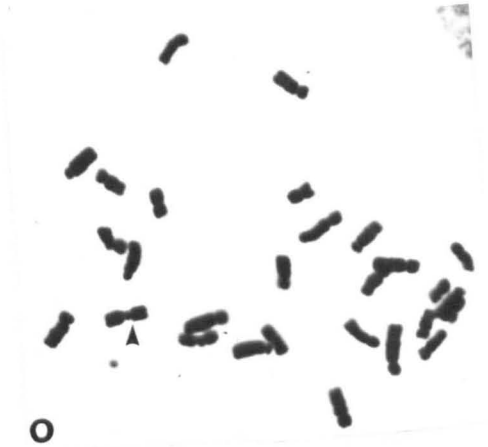
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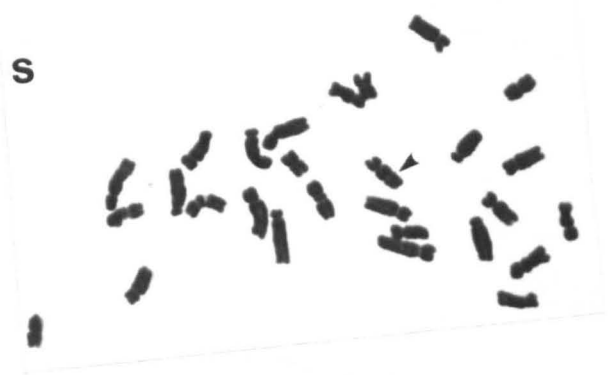
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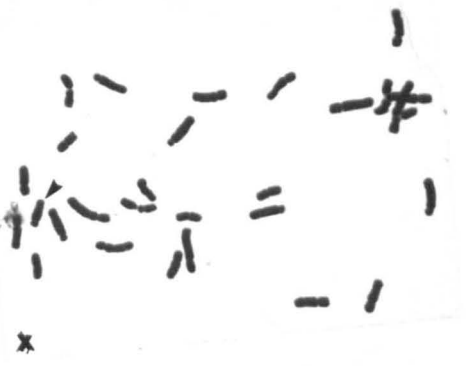
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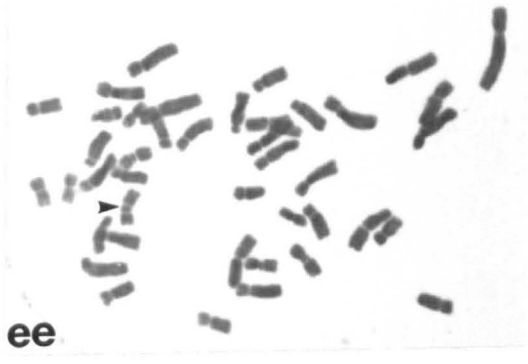
ee-hh Hexaploids



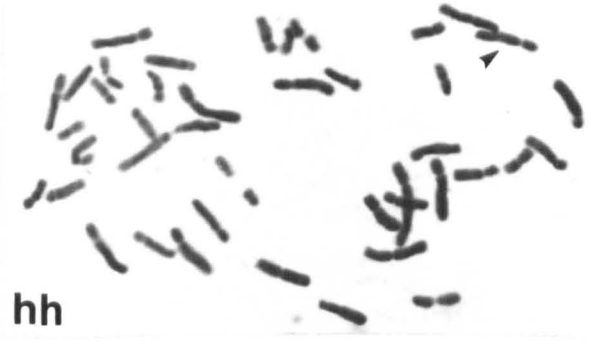



Plate 5.7 Unique duplications

a MS6 (diploid)

b-n Duplications in tetraploids

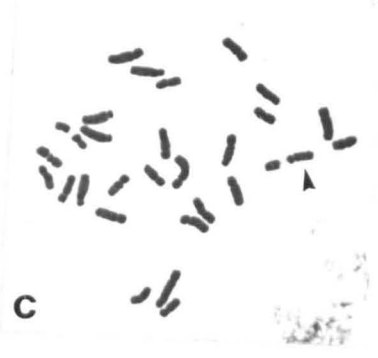
| | | |
|---|---|---|
| b | GN32 | B1 duplication |
| c | PSG29 | B3 deletion |
| d | AS18 | B3 duplication |
| e | AS15 | B3 duplication |
| f | GN28 | B3 and B5 duplications |
| g | PDA5 | B3 duplication |
| h | PP26 | B3 duplication |
| i | PH6 | B3 duplication |
| j | AS13 | B3 duplication |
| k | AS22 | B3 duplication |
| l | PH24 | B3 duplication |
| m | SSL3 | B3 and B6 duplications |
| n | ADC17 | B4 duplication |

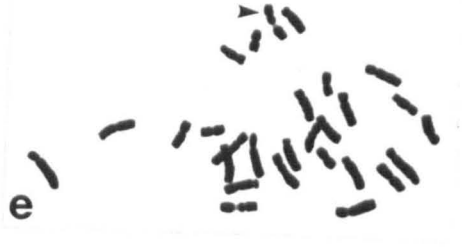
o-q Duplications in hexaploids

| | | |
|---|---|---|
| o | CT28 | B1 duplication |
| p | GD22 | B3 duplication |
| q | RT48 | A5 duplication and A7 deletion |

a Diploid


b-n Tetraploids



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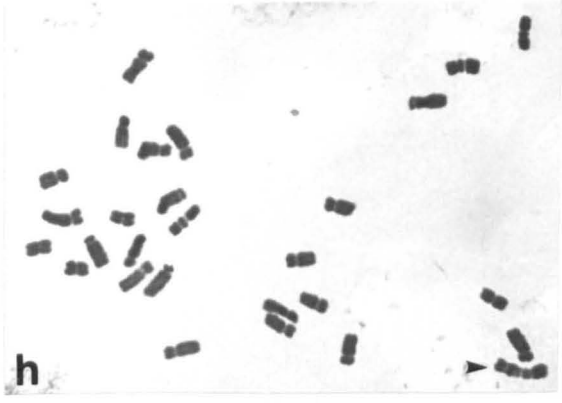
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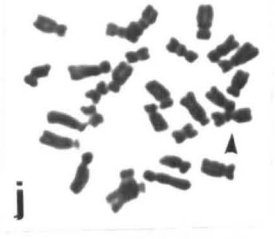
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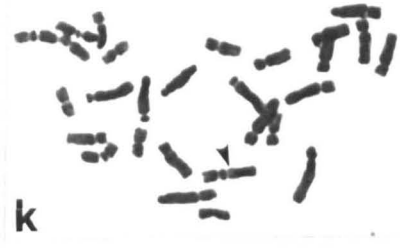
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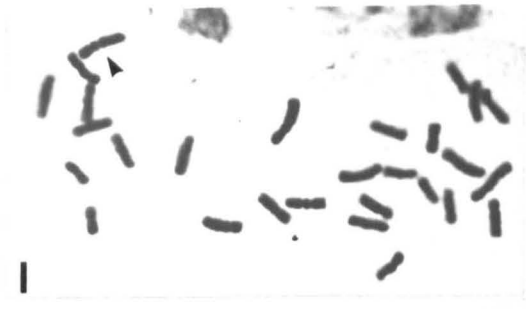
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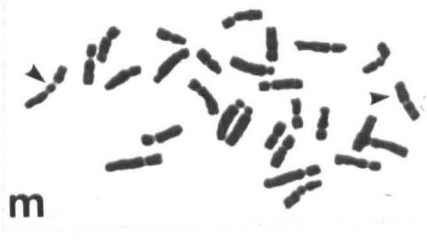
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n

o-q Hexaploids



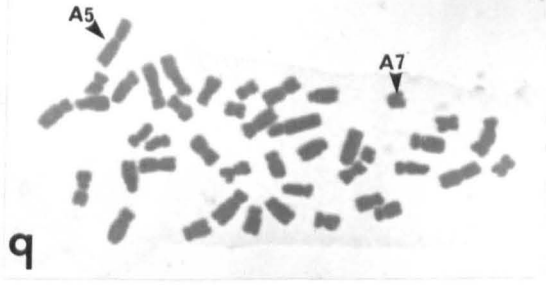


Plate 5.8 Unique interchanges

(a) GP7 $B2^{L}/B7^{L}$ interchange

(b) HC21 $B4^{L}/B6^{S}$ interchange

(c) FR16 $B1^{L}/B6^{L}$ interchange

Plate 5.9 Interphase micronuclei in root tip cells of PH1

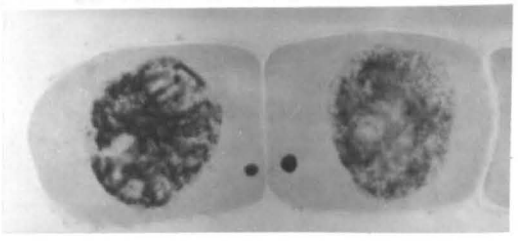
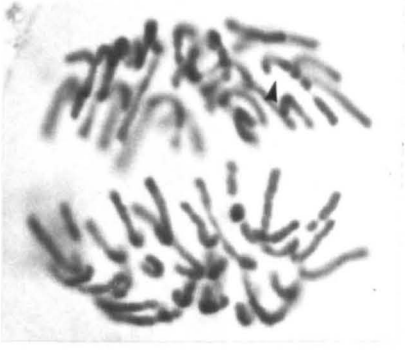


Plate 5.10 Mitotic anaphase in PP26. Duplication chromosome arrowed



b) Tetraploids

Twenty-six of the 35 tetraploid populations contained plants with unique structural rearrangements. In total, 67 such plants were detected, including 34 plants which also carried polymorphic variant chromosomes (Table 5.6). The overall level of unique structural variation in tetraploid plants was 5.5%, so that on average, one or two unique variants were found in a population sample of about 30 plants (Table 5.6).

Barras Nose

BN10 Pollen stainability 99%

A pericentric inversion in a B5 chromosome moved the centromere to a more median position, changing the arm ratio from 1:2.24 to 1:1.15 (Fig. 5.2; Plate 5.6w).

Pentire Head

PH6

PH6 contained a B3 chromosome with a long arm duplication of 0.5 μm (Fig. 5.3; Plate 5.7i).

PH24

The distal segment of the long arm of a B3 chromosome in PH 24 was increased by 1.8 μm (Fig. 5.3; Plate 5.7l).

Cow and Calf

C & C 8 Pollen stainability 98%

A single B7 chromosome with a 0.8 μm deletion was observed in C & C 8 (Fig. 5.1). This plant also carried a polymorphic pericentric inversion B3 chromosome (Plate 5.5r).

C & C 24

C & C 24 carried a B3 chromosome lacking the entire segment of the long arm distal to the NOR (2.95 μm) (Fig. 5.1; Plate $5.5_o$).

Gara Point

GP7 Pollen stainability 99%

GP7 was apparently heterozygous for an interchange between the long arms of B2 and B7, with B7 increased and B2 decreased in length (Plate 5.8a). There is no meiotic evidence for interchange but the presence of equal sized deletion and duplication is less likely.

Prawle Point

PP26 Pollen stainability 85%

This plant carried a long arm duplication of B3. The duplicated segment of the novel chromosome included two additional constrictions, and increased the B3 length by 3.0 μm (45%) (Fig. 5.3; Plate 5.7h). This duplication may have arisen by interchange between two B3 chromosomes. Break points would then be distal to the NOR in one chromosome and immediately proximal to the centromere in the short arm of the other. The dicentric product of reunion would then have the required number of constrictions. However, at anaphase the chromosome is clearly monocentric (Plate 5.10). If the break has occurred through the centromere then activity may have been lost.

Berry Head

BH31

This plant carried a single B1 deletion chromosome which lacked 2.2 μm (50%) of the short arm (Fig. 5.1). In addition, the plant was pentasomic for B2, simplex for a polymorphic B1 duplication and duplex for polymorphic inversion 3-1 (Plate 5.5a).

BH37

This plant carried a nucleolar-organiser chromosome (B3) inversion. The rearrangement is complex requiring at least two overlapping inversions since all three identifiable regions of the novel B3 differ from the

standard (Fig. 5.2; Plate 5.6 ). A B3 pericentric inversion (Inv 3-1) which reaches polymorphic proportions in this population has a short arm of equal length to that of the novel B3 in BH37 (Plate 6.2 d-i). This novel B3 then may be a paracentric inversion derivative of Inv 3-1.

BH49 Pollen stainability 100%

The unique chromosome of BH49 was a B2 with a long arm deletion which reduced the length by 0.9 μm (Fig. 5.1). BH49 was also duplex for the polymorphic inversion Inv 3-1 and simplex for polymorphic duplication Dup 1-1 (Plate 5.5e).

Long Quarry Point

LQP2 Pollen stainability 89%

LQP2 carried a B2 with a long arm deletion which reduced the length of the arm by 3.35 μm (55%) (Fig. 5.1; Plate 5.5l).

LQP10

In LQP10 two possibilities for the origin of the unique chromosome must be considered. The unique B1 chromosome has a short arm of 3.6 μm, the same length as that of polymorphic duplication Dup 1-1 which reaches a high frequency in this population. The long arm then may have a deletion of approximately 0.9 μm (Fig. 5.1). An alternative explanation is that this chromosome results from a pericentric inversion. There is no meiotic evidence to differentiate these possibilities. LQP10 also carries a B3 pericentric inversion chromosome (Inv 3-1) and a B1 duplication (Dup 1-1) (Plate 5.5b).

Isle of Wight

IOW11

IOW11 contained a B3 chromosome with a unique pericentric inversion (Fig. 5.2; Plate 5.6i). The break points were in the short arm and the

centromere - NO region giving an arm ratio of 1:2.28. In this plant four NORs were not always expressed.

Hampton Court

HC1

A B3 chromosome in HC1 had a short arm reduced by 0.5 μm (23%) (Fig. 5.1; Plate 5.5m).

HC15

HC15 carried a B2 chromosome with a deletion which reduced the length of the long arm by 0.5 μm (42%) (Fig. 5.1). In addition the individual was simplex for the polymorphic inversion Inv 3-1 (Plate 5.5i).

HC21

HC21 contained two unique structurally rearranged chromosomes possibly as a result of reciprocal interchange. The long arm of a B4 was of increased length while the short arm of B6 was reduced by a similar amount (Plate 5.8b). There is, however, no meiotic evidence for interchange although this is the simplest explanation.

HC25

The unique variant in HC25 was a B2 chromosome with a long arm deletion. The long arm was reduced by 2.9 μm (47%) (Fig. 5.1; Plate 5.5k).

Grosnez Point

GN5 Pollen stainability 100%

GN5 contains a B3 in which virtually the entire short arm has been deleted (2.1 μm; 88%; Fig. 5.1). This plant was also simplex for polymorphic inversion Inv 3-1 (Plate 5.5n).

GN28 Pollen stainability 92%

Two unique variant chromosomes were present in this plant (Plate 5.7f). It carries a B3 with a duplication which doubles the centromere - nucleolar

organiser distance and a B5 with a long arm duplication which increases the length by 2.4 µm (Fig. 5.3). In addition, GN28 was simplex for a polymorphic B6 pericentric inversion (Inv 6-1).

GN32 Pollen stainability 96%

A single copy of B1 had a short arm increased by 1.7 µm (69%) over standard (Fig. 5.3) The plant was also duplex for polymorphic inversion 3-1 (Plate 5.7b).

Corbiere

CB8

One usually metacentric B7 carried a pericentric inversion which altered the arm ratio to 1:1.43 (Fig. 5.2). In addition, the complement contained one copy of the polymorphic long arm duplication of B1 (Dup 1-3) (Plate 5.6 aa).

CB12

CB12 was simplex for a B3 pericentric inversion (Fig. 5.2). The breakpoints can be pinpointed exactly since one is located in the NOR itself, and the other in the short arm 1.3 µm from the centromere. An additional small secondary constriction can be seen in the short arm (Plate 5.6n).

CB16

A single B6 chromosome was present with a deletion of 1.3 µm (66%) of the short arm (Fig. 5.1; Plate 5.5q).

Fort Regent

FR16

Two unique chromosomes were present in the complement of FR16: a B1 with an increased long arm and a B6 with a reduced long arm (Plate 5.8c). The deviations from the standard chromosome were 1.5 µm in each case and

so a reciprocal interchange would explain the results. An alternative is that we have independent duplication and deletion events in the generation of these unique variants. This population is polymorphic for a long arm duplication of B1 which is of identical morphology. Meiotic analysis is required to resolve this.

FR28

In a B3 chromosome of FR28 all three identifiable regions differed in length from the standard type (Fig. 5.2). The generation of this inversion chromosome, then, requires more than a two-break event. The short arm is reduced by 0.3 μm, the centromere - NOR segment increased by 1.0 μm, and the distal segment of the long arm reduced by 0.9 μm. FR28 was also simplex for a polymorphic pericentric B6 inversion (Inv 6-1)(Plate 5.6s).

Fort Corbelets

FC26

In FC26 (Plate 5.5d) a short arm deletion of a B2 chromosome reduced the arm length by 0.7 μm (59%) (Fig. 5.1).

FC27

FC27 (Plate 5.6k) carried a B3 chromosome with a pericentric inversion (Fig. 5.2). The breakpoints were in the short arm and the centromere - NOR segment, reducing the length of the former and increasing the latter by 0.3 μm. This plant was also pentasomic for B5.

Cap Carteret

CC 28

The variant chromosome resulted from a pericentric inversion in B1 which shifted the centromere to a more markedly acrocentric location (Fig. 5.2). The new arm ratio is 1:5.44. Remarkably, half the cells in both roots included a metacentric 6.2 μm in length and were deficient for a

B7 (Plate 5.6c). The rest of the complement was normal. No simple origin of this metacentric can be proposed since both arms are longer than B7.

Saint Georges de la Rivière

SG2/11

SG2/11 contained a B3 chromosome in which a pericentric inversion shifted the centromere to give an arm ratio of 1:2.04 (Fig. 5.2).

SG6/7

A single B2 chromosome in SG6/7 had a long arm reduced by 2.1 μm (34.2%) (Fig. 5.1). Remarkably, the plant was pentasomic for B1, B3 and B6, presumably as a result of a backcross between a tetraploid and an autopentaploid (Plate 5.5h).

Mon Griffon

MG29 Pollen stainability 0%

This plant carried a B3 chromosome which had undergone pericentric inversion. The variant chromosome is more acrocentric than the standard with an arm ratio of 1:2.19 (Fig. 5.2). MG29 was also duplex for polymorphic inversion 6-1 (Plate 5.6l).

Les Hougues

LH19

The long arm of a single B2 chromosome in LH19 was reduced by 2.4 μm (39.8%) (Fig. 5.1; Plate 5.5j).

Bréville-sur-mer

BSM5

A B2 chromosome of standard length in BSM5 (Plate 5.6f) was telocentric, possibly as a result of pericentric inversion (Fig. 5.2). One of the break points would, therefore, be through the telomere, and the other

proximal to the centromere. In addition, the plant was simplex for inversion 3-1, duplex for inversion 6-1 and simplex for duplication 1-2, all polymorphic in this population.

BSM31

This plant carried a B7 with a 1.2 μm (50%) deletion of one arm (Fig. 5.1, Plate 5.5s).

Le Verger

LV28

The variant chromosome was a B2 with a pericentric inversion which shifted the centromere to a more median position (Fig. 5.2; Plate 5.6g).

Plage de Guen

PDG3

This plant contained a B3 chromosome with a paracentric inversion. The inversion shifted the NOR to a more proximal position by 0.6 μm (Fig. 5.2). The plant was also simplex for the polymorphic duplication Dup 3-10 (Plate 5.6p).

Pointe du Percho

PDP5

In PDP5 (Plate 5.6o) the variant chromosome was a unique B3 pericentric inversion (Fig. 5.2). The centric shift gave a more metacentric chromosome with an arm ratio of 1:1.43. In addition, PDP5 was simplex for polymorphic duplication Dup 1-2.

PDP10

A single B3 chromosome carried a pericentric inversion which shifted the NOR to a more distal location by 0.25 μm (Fig. 5.2). PDP10 was also simplex for the polymorphic duplication Dup 1-2 (Plate 5.6q).

PDP14

PDP14 (Plate 5.6e) contained two distinct unique variants. A pericentric inversion in a B1 chromosome shifts the centromere position to a slightly more median position (Fig. 5.2). One B3 carries a short arm duplication 0.8 µm (35%) in length (Fig. 5.3).

PDP27

A chromosome 7 in PDP27 (Plate 5.6dd) carries a pericentric inversion which shifts the centromere to an acrocentric position (Fig. 5.2).

Pointe de St. Gildas

PSG29 Pollen stainability 61%

The unique variant B3 chromosome in PSG29 carries a short arm duplication of 1.9 µm (79%) (Fig. 5.3). In addition PSG29 was simplex for the polymorphic pericentric inversion Inv 3-1 (Plate 5.7c).

Sion sur l'Oceon

SSL3 Pollen stainability 89%

SSL3 carried two unique chromosomes (Plate 5.7m). A B6 chromosome had a short arm 1.5 µm (75%) longer than standard giving an almost metacentric chromosome (Fig. 5.3). A B7, normally metacentric, had undergone pericentric inversion to give a markedly acrocentric chromosome (Fig. 5.2; arm ratio 1:1.56).

SSL5

A chromosome B3 in this plant showed considerable rearrangement. The centromere was shifted to a more median position and the NOR, normally interstitial in the long arm, is now terminal on the short arm (Fig. 5.2; Plate 5.6u).

SSL16 Pollen stainability 71%

The variant B3 chromosome in this plant had a NOR shifted distally by 0.9 μm as a result of a long arm paracentric inversion. There is a faint constriction at the normal N.O. site perhaps indicating that one break point occurred at the proximal end of the N.O. region itself. If this is the case, then the new position of the N.O. in the inverted chromosome marks the second break point (Fig. 5.2; Plate 5.6r).

SSL34

A chromosome B5 in this plant was reduced by 3.2 μm (70%) in the long arm giving an arm ratio of 1:1.46 (Fig. 5.1; Plate 5.5p).

Anse de Cayola

ADC10

ADC10 (Plate 5.6z) contained a B6 chromosome with a more median centromere as a result of pericentric inversion (Fig. 5.2).

ADC16

A normally metacentric B7 in this plant had undergone pericentric inversion, shifting the centromere to a more acrocentric location (Fig. 5.2; Plate 5.6bb).

ADC17

This plant contained a B4 chromosome with a 0.7 μm (29%) duplication of the short arm (Fig. 5.3). This plant was also simplex for the polymorphic inversion 3-1 (Plate 5.7n).

ADC19

In this plant a B2 chromosome with a reduced long arm was found (Fig. 5.1; Plate 5.5f). The deletion was 1.0 μm (16% of the length of the arm). Additionally, a polymorphic duplication chromosome was

present (Dup 1-2). The expression of the NORs was variable with only three visible in some cells.

ADC29

This plant carried a B6 chromosome with a pericentric inversion shifting the centromere to a much more acrocentric position (Fig. 5.2; Plate 5.6x). In addition, ADC29 was also an aneuploid with 29 chromosomes, pentasomic for B2.

Argentré sur Jouanne

ASJ2 Pollen stainability 74%

The variant chromosome in ASJ2 resulted from pericentric inversion in B4 (Fig. 5.2). The centromere was shifted to a more acrocentric position. Interestingly, this was the only inversion observed in chromosome B4, at any ploidy level. In addition, the plant was simplex for inversion 3-8 and duplex for deletion 5-1, both polymorphic in this population (Plate 5.6v).

ASJ20

In ASJ20 (Plate 5.6d), two B1 chromosomes were unique, both resulting from pericentric inversion. The inversions resulted in one chromosome more acrocentric, with an arm ratio of 1:4.12, and the other less acrocentric with an arm ratio 1:2.41 (standard B1 = 1:2.55) (Fig. 5.2).

ASJ31

The variant chromosome in this plant, a B2, has lost 2.6 μm (43%) from the long arm (Fig. 5.1).

Pont de l'Argenton

PDA1

PDA1 (Plate 5.6cc) carried an acrocentric B7 chromosome as a result of pericentric inversion. The arm ratio of the novel chromosome was 1:1.26 (Fig. 5.2). In addition PDA1 was simplex for polymorphic inversion 1-1 and polymorphic duplication 4-1.

PDA5 Pollen stainability 94%

A nucleolar-organiser chromosome B3 in this plant had a long arm increased by 0.4 µm. The additional material was apparently inserted into the N.O. region itself, dividing the constriction into two segments (Fig. 5.3). The plant was also simplex for the polymorphic inversion chromosome Inv 1-1, and carried seven B-chromosomes ($6B^{st-2} + B^{st-3}$) (Plate 5.7g).

PDA29

In PDA29 (Plate 5.5c) one B1 chromosome had a short arm 1.2 µm (48%) shorter than standard (Fig. 5.1). PDA29 was also simplex for two polymorphic inversions, Inv 1-1 and Inv 3-1, and carried two B chromosomes ($2B^{st-1}$).

Aghios Stephanos

AS10

AS10 (Plate 5.6y) contained a B6 chromosome with a pericentric inversion which shifted the centromere to a more acrocentric location giving an arm ratio of 1:3.33 (Fig. 5.2).

AS13

A single B3 chromosome in this plant carried a duplication which increased the long arm distal to the N.O. region by 0.6 µm (20%) (Fig. 5.3). In addition AS13 was simplex for inversion chromosome 3-6,

polymorphic in this population (Plate 5.7j).

AS14

This plant contained two distinct variant B3 chromosomes, both pericentric inversions (Fig. 5.2). In one, the long arm break point was in the segment between centromere and NOR and the resultant chromosome was more metacentric. In the other the long arm break point was distal to the NOR but the resultant chromosome was also more metacentric. In addition the plant was simplex for two duplications - Dup 5-2 and Dup 6-1 - polymorphic in the AS population (Plate 5.6h).

AS15

In the variant B3 chromosome in AS15 the centromere - NOR distance was increased by 0.8 μm (70% of the long arm) (Fig. 5.3). Remarkably AS15 was also duplex for two polymorphic duplications, Dup 5-2 and 6-1 (Plate 5.7e).

AS18

The unique chromosome in this plant was a B3 in which the centromere - NOR distance was increased by 0.4 μm (Fig. 5.3; Plate 5.7d).

AS22

A chromosome B3 in AS22 carried a duplication in which the distal part of the long arm was increased by 1.5 μm (Fig. 5.3). In addition, AS22 was simplex for two polymorphic chromosomes, Inversion 3-7 and duplication 5-2 (Plate 5.7k).

Kayos

KV3

Two of the four B2 chromosomes in KV3 are unique deletion types. In one, the long arm is reduced by 1.1 μm (19%) and in the other by

2.6 µm (43%) (Fig. 5.1). In addition, KV3 was simplex for the polymorphic duplication chromosome 3-5 (Plate 5.5g).

KV15

Two unique variant chromosomes were present in KV15 (Plate 5.6m). A B3 resulted from pericentric inversion with break points in the centromere - NOR region and short arm. The centromere was shifted to a more acrocentric position (Fig. 5.2). Secondly, a B2 chromosome had a short arm reduced by 0.8 µm (70%) (Fig. 5.1). A single B-chromosome ($B^{st-4}$) was also present in KV15.

KV25

In KV25 (Plate 5.6j) a B3 chromosome was more acrocentric as a result of pericentric inversion (Fig. 5.2). The break points were in the short arm and the centromere - NOR segment. A single copy of the polymorphic duplication chromosome 6-1 was also present.

c) Hexaploids

Cudden Point

CP7

An A6 chromosome in CP7 had a short arm reduced in length by 3.1 µm (77%) (Fig. 5.1; Plate 5.5aa).

CP34 Pollen stainability 89%

In this plant a B3 chromosome had the centromere in a more acrocentric position as a result of pericentric inversion (Fig. 5.2; Plate 5.6ee).

CP38

Two unique variants were present in this complement; a pericentric inversion in A6 which shifted the centromere to a more acrocentric position (Fig. 5.2) and a B4 duplication which increases the length of

the short arm by 0.6 μm (Fig. 5.3; Plate 5.6hh).

Caerleon Cove

CT1 Pollen stainability 74%

In plant CT1 the long arm of a B3 chromosome was reduced by 2.95 μm (Fig. 5.1). In addition the plant was simplex for the polymorphic inversion chromosome B 3-1.

CT3

A unique B5 chromosome deletion in CT3 reduced the long arm by 2.5 μm (Fig. 5.1).

CT10

A B7 chromosome in this plant was represented by a telocentric element of half the standard length (Fig. 5.1). In addition, the plant was monosomic for A7 (Plate 5.5z).

CT15

Plant CT15 carried a deleted B1 chromosome in which the long arm was reduced by 2.7 μm (43.2%) (Fig. 5.1). The plant was also simplex for polymorphic inversion chromosome 3-1.

CT28

The unique variant in CT28 was a B1 chromosome with a long arm duplication of 1.4 μm (22%) (Fig. 5.3). CT28 was also duplex for polymorphic inversion chromosome B 3-1 (Plate 5.7o).

Goonhilly Downs

GD6 Pollen stainability 85%

GD6, an aneuploid with 2n = 43 contained a B7 chromosome with a deletion. The size of the deletion is not known (Fig. 5.1).

GD22 Pollen stainability 85%

This plant carried a B3 with a 1.3 μm duplication of the distal segment of the long arm (Fig. 5.3: Plate 5.7p).

Rill Top

RT6

An A3 chromosome was present as a metacentric in RT6 as a result of pericentric inversion (Fig. 5.2; Plate 5.6gg).

RT7

A B3 chromosome in RT7 (Plate 5.5w) had a centromere - NOR region reduced by 0.5 μm from standard (Fig. 5.1).

The plant was also simplex for polymorphic duplication chromosome B 3-8 and in addition was aneuploid with 2n = 43. The additional chromosome was a novel acrocentric 2.1 μm in length. This is about half the length of the smallest chromosome in the complement (B7).

RT33

This plant contained two unique deletion chromosomes. A deletion in B1 reduced the length of the long arm by 3.7 μm (61%) and in B3 the whole of the short arm (2.4 μm) was absent giving a telocentric (Fig 5.1; Plate 5.5t).

RT48 Pollen stainability 64%

Two unique variants were present in the complement of RT48, a duplication and a deletion chromosome (Plate 5.7q). An A5 chromosome had a short arm increased by 3.3 μm (55%) (Fig. 5.3), and an A7 with a long arm reduced by 4.7 μm (72%) Fig. 5.1). RT48 was mitotically unstable and micronuclei were observed in many interphase cells. In addition, several metaphase complements were shattered into many fragments (Plate 5.1 ).

L'Eree

LE28

LE28 (Plate 5.6ff) contained a nearly-telocentric B5 chromosome and a more metacentric B3, both as a result of pericentric inversion (Fig. 5.2). In the latter the break points were in the short arm and centromere - NOR segment of the long arm. In addition the plant was simplex for polymorphic inversion chromosome B 3-1.

St. Martin's Point

SMP3

A B3 chromosome in the complement of SMP3 was represented by a short arm telocentric (Fig. 5.1). This plant was also numerically variable with a B7 chromosome absent from some cells.

SMP9

In a single B3 chromosome the centromere - NOR distance was reduced by 0.5 μm (Fig. 5.1). SMP9 was trisomic for chromosome A7 (Plate 5.5v).

Les 1'Aches

LL14 Pollen stainability 94%

LL14 contained two unique chromosomes (Plate 5.5x). In one B3 chromosome the distal segment of the long arm was reduced by 0.6 μm (Fig. 5.1) while in another the position of the NOR was shifted proximally by 0.4 μm as a result of paracentric inversion (Fig. 5.2).

A third B3 in the complement was a B 3-9 inversion chromosome, polymorphic in this population. LL14 was, additionally, trisomic for B2 (2n = 41).

LL18 Pollen stainability 99%

A B3 chromosome in this plant was 0.8 μm shorter than standard. The

deletion affected the distal portion of the long arm (Fig. 5.2). The short arm and centromere - NOR lengths of this chromosome are identical to those in inversion chromosome B 3-1 (Plate 5.5y).

LL30 Pollen stainability 99%

A unique B3 chromosome in LL30 was characterised by a reduction of 0.5 μm (12%) in the centromere - NOR distance (Fig. 5.1). LL30 was numerically variable with an A7 chromosome absent from some cells (Plate 5.5u).

The chromosome specificity of structural rearrangements

a) Deletion

At the cellular level of spontaneous change in root tip mitoses the distribution of deletions amongst the B genome chromosomes is proportional to chromosome length ($\chi^2 = 8.84$, $P > 0.2$; Table 5.4). Deletions affecting whole plants are clearly distributed non-randomly between the B-genome chromosomes ($\chi^2 = 33.29$, $P < 0.001$; Table 5.7). This may be evidence of selection, but there is no evidence that the incidence of deletions is random in gametic chromosomes.

The majority of the $\chi^2$ value is made up by an excess of deletions affecting chromosomes B2 and B3 and a deficit in B4.

Deletions affecting the long arm of B2 gave chromosomes in which the arm remaining varied between 90% and 15% of its original length (see Fig. 5.1). Deletions smaller than 10% are likely to remain undetected.

Two deletions affecting the short arm of B2 were found though this arm is by far the shortest in the complement (1.18 μm). The 13 deletions of chromosome 3 affected all three parts of the chromosome. Five reduced the length of the short arm, three the centromere - NOR distance and

Table 5.6 Whole-plant numerical and structural variation in diploid, tetraploid and hexaploid populations of S. autumnalis. Numerical variants are those plants with the majority of cells scored deviating from the standard number

| Population | | No. plants | No. with normal karyotype | No. of numerical variants | Structural variants | | | Total | No. of different polymorphisms | Total chromosomes affected % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Unique | Polymorphic | Unique/ polymorphic | | | |
| 2x | LK | 24 | 19 | - | - | 3 | 1 | 4 | 2 | 1.5 |
| | MS | 27 | 15 | - | 1 | 10 | 1 | 12 | 2 | 3.4 |
| | PK | 30 | 14 | 1* | 1 | 14 | 1 | 16 | 3 | 4.5 |
| Total | | 81 | 48 (59.2%) | 1 (1.3%) | 2 (2.5%) | 27 (33.3%) | 3 (3.7%) | 32 (39.5%) | | 3.3 |
| 4x | MP | 28 | 15 | 1 | - | 12 | - | 12 | 1 | 2.2 |
| | BN | 32 | 19 | - | 1 | 12 | - | 13 | 1 | 1.9 |
| | PH | 30 | 18 | - | 2 | 10 | - | 12 | 1 | 1.3 |
| | C+C | 34 | 16 | 1 | 1 | 15 | 1 | 17 | 1 | 2.2 |
| | GP | 29 | 25 | - | 2 | 2 | - | 4 | 1 | 0.6 |
| | GR | 32 | 28 | - | - | 4 | - | 4 | 1 | 0.5 |
| | PP | 32 | 10 | - | - | 21 | 1 | 22 | 2 | 3.4 |
| | IC | 33 | 26 | 2 | - | 7 | - | 7 | 1 | 1.0 |
| | BH | 53 | 13 | 1 | 1 | 37 | 2 | 40 | 2 | 4.5 |
| | LQP | 32 | 10 | - | 1 | 20 | 1 | 22 | 2 | 3.8 |
| | IOW | 36 | 32 | - | 1 | 3 | - | 4 | 2 | 0.4 |
| | HC | 30 | 26 | - | 3 | - | 1 | 4 | 1 | 0.7 |
| | GN | 30 | 8 | - | - | 20 | 3 | 22 | 2 | 4.3 |
| | CB | 31 | 18 | 2 | 2 | 15 | 1 | 18 | 6 | 1.3 |
| | FR | 30 | 12 | 6 | - | 13 | 2 | 15 | 4 | 2.4 |
| | VP | 31 | 4 | 2 | - | 27 | - | 27 | 5 | 4.9 |
| | FC | 30 | 12 | 1 | 2 | 16 | - | 18 | 2 | 2.7 |

* centric fission

/continued

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Table 5.6 continued

| Population | | No. plants | No. with normal karyotype | No. of numerical variants | Structural variants | | | Total | No. of different polymorphisms | Total chromosomes affected % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Unique | Polymorphic | Unique/ polymorphic | | | |
| | LM | 7 | 2 | - | - | 5 | - | 5 | 1 | 3.1 |
| | CCF | 30 | 20 | - | 1 | 9 | - | 10 | 1 | 1.2 |
| | SG | 151 | 94 | 8 | 2 | 50 | - | 52 | 5 | 1.4 |
| | MG | 30 | 13 | 2 | - | 16 | 1 | 17 | 2 | 2.6 |
| | LH | 27 | 16 | 2 | 1 | 9 | - | 10 | 2 | 1.5 |
| | BSM | 31 | 6 | - | 1 | 23 | 1 | 25 | 3 | 5.0 |
| | LV | 29 | 16 | 2 | 1 | 10 | - | 11 | 5 | 1.6 |
| | PDG | 26 | 17 | 1 | - | 8 | 1 | 9 | 3 | 1.5 |
| | PDT | 30 | 20 | 1 | - | 9 | - | 9 | 3 | 1.7 |
| | PDP | 30 | 19 | - | 2 | 7 | 2 | 11 | 3 | 2.0 |
| | PAL | 22 | 16 | 1 | - | 5 | - | 5 | 3 | 1.0 |
| | PSG | 24 | 21 | 1 | - | 1 | 1 | 2 | 3 | 0.6 |
| | SSL | 21 | 21 | 1 | 4 | 6 | - | 10 | 3 | 1.5 |
| | ADC | 28 | 19 | 2 | 3 | 3 | 2 | 8 | 3 | 1.3 |
| | ASJ | 32 | 18 | 2 | 3 | 9 | 2 | 14 | 4 | 2.3 |
| | PDA | 36 | 15 | - | - | 9 | 3 | 12 | 4 | 2.0 |
| | AS | 23 | 3 | 1 | 2 | 14 | 4 | 20 | 7 | 5.6 |
| | KV | 23 | 10 | - | 1 | 9 | 2 | 12 | 5 | 3.9 |
| Total | | 1163 | 638 (54.9%) | 40 (3.4%) | 37 (3.2%) | 436 (37.5%) | 30 (2.6%) | 503 (43.3%) | | 2.21 |
| 6x | CP | 34 | 27 | 4 | 3 | - | - | 3 | - | 0.3 |
| | CT | 30 | 13 | 7 | 3 | 6 | 3 | 12 | 1 | 1.4 |
| | GD | 31 | 22 | 8 | 2 | - | - | 2 | - | 0.2 |
| | RT | 35 | 20 | 6 | 3 | 8 | 1 | 12 | 3 | 1.1 |
| | LC | 28 | 5 | 7 | - | 20 | - | 20 | 2 | 2.0 |
| | LE | 28 | 11 | 3 | - | 15 | 1 | 16 | 1 | 2.1 |
| | SMP | 31 | 10 | 7 | 1 | 16 | 1 | 18 | 1 | 1.8 |
| | LL | 29 | 11 | 7 | 1 | 13 | 2 | 16 | 3 | 1.7 |
| Total | | 246 | 119 (48.4%) | 49 (19.9%) | 13 (5.3%) | 78 (31.7%) | 8 (3.3%) | 99 (40.2%) | | 1.3 |

Table 5.7 The numbers of whole plant structural changes (unique and polymorphic) affecting the different B genome chromosome groups in S. autumnalis. Numbers of polymorphic changes are in parentheses. Expected numbers calculated on the basis of chromosome length.

| | | B1 | B2 | B3 | B4 | B5 | B6 | B7 | Total | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Deletions | obs. | 5 | 15 | 13 | - | 3(1) | 1 | 3 | 40(1) | 33.29 | <0.001 |
| | | | | | | | | 4 | | | |
| | exp. | 7.33 | 6.23 | 5.56 | 6.15 | 5.60 | 4.93 | 4.21 | | | |
| | | | | | | | | 9.14 | | | |
| Inversions | obs. | 11(4) | 3(1) | 27(9)* | 1 | 4(1) | 6(2) | 9(3) | 61(20) | 56.22 | <0.001 |
| | exp. | 11.17 | 9.50 | 8.47 | 9.37 | 8.54 | 7.52 | 6.42 | | | |
| Duplications | obs. | 9(6) | 1(1) | 22(9) | 3(1) | 2(2) | 2(1) | 2(1) | 42(21) | 57.44 | <0.001 |
| | | | | | | | | 4 | | | |
| | exp. | 7.69 | 6.54 | 5.83 | 6.45 | 5.88 | 5.18 | 4.42 | | | |
| | | | | | | | | 9.60 | | | |

*Excluding paracentric inversions

five the long arm distal to the NOR region. Two breaks through the centromere and two through the nucleolar organiser were also recorded.

Chromosome B4 was unique in that no deletions for this chromosome were recorded. It may be that deletions for B4 are lethal.

There is a significant difference in the distribution of deletions between spontaneous cellular mutations and whole-plant events ($\chi^2 = 10.38$, $P < 0.05$; Table 5.8) which suggests that selection operates against structural changes in some chromosomes. However, it is necessary to compare the production of structural changes at meiosis with change affecting whole plants before the action of selection can be unequivocally demonstrated. This presupposes that changes in meiosis are non random.

b) Interchange

The distribution of spontaneous interchanges at the cellular level is proportional to chromosome length ($\chi^2 = 0.76$, $P > 0.9$; Table 5.4).

c) Inversion

Pericentric inversions affecting whole plants were not distributed between the B genome chromosomes in relation to chromosome length ($\chi^2 = 56.22$; $P < 0.001$, Table 5.7). The greater part of the deviation from the expected results from chromosome groups B2, 3 and 4. The same pattern emerges for groups B3 and B4, which, for inversions as for deletions, contain more than the expected number. Twenty-seven pericentric inversions were in chromosome 3 alone, over three times greater than the expected number of 8.47. Chromosomes B2 and B4 were both deficient in inversions with 3 and 1 respectively, the expected numbers being 9.50 and 9.37. Pericentric inversions would not be expected to be very frequent in B2 because of the subtelocentric nature of this chromosome, with a short arm of 1.18 μm. The rarity of inversions in B4 cannot be explained in these terms and indicates,

Table 5.8 Comparison of the incidence of spontaneous and whole plant deletions affecting B genome chromosomes in S. autumnalis. Expected numbers, calculated on the basis of chromosome length, are in parentheses.

| Type of Deletion | B1 | B2 | B3 | B4 | B5 | B6 | B7 | Total |
|---|---|---|---|---|---|---|---|---|
| Spontaneous | 9 | 14 | 17 | 10 | 7 | 7 | 10 | 74 |
| | | | | | 34 | | | |
| | (9.17) | (18.7) | (19.3) | | (26) | | | |
| Whole plant | 5 | 15 | 13 | 0 | 3 | 1 | 3 | 40 |
| | | | | | 7 | | | |
| | (5.3) | (10.3) | (10.7) | | (14.6) | | | |
| Total | 14 | 29 | 30 | 10 | 10 | 8 | 13 | 114 |

$\chi^2_{(3)} = 10.38$ ($P < 0.05$)

with the evidence from deletions, that plants with variant chromosome 4 are selected against. The nucleolar-organiser chromosome seems particularly susceptible to inversion although with two markers on the chromosome inversions may be more readily detectable. In Allium schoenoprasum (Bougourd, 1977) all three pericentric inversions affected the nucleolar organiser chromosome. Nucleolar chromosomes in relation to structural aberrations will be discussed in more detail later.

d) Duplication

The incidence of duplication affecting whole plants was not related to chromosome length ($\chi^2$ = 57.44, P < 0.001; Table 5.7). Chromosome B2 had fewer duplications than would be expected (1 only as opposed to 6.54) while B3 duplications (22) accounted for over half the total number of 42. This high frequency of B3 duplication may in part be explained by the morphology of the chromosome since the additional marker provided by the nucleolar-organiser makes chromosome deviants more noticeable. The incidence of duplication amongst chromosomes 1, 4, 5, 6 and 7 was approximately in proportion to the expected numbers.

## Discussion

### The nature and extent of somatic chromosome breakage

Spontaneous chromosome breakage occurs with a low frequency in all organisms. In Scilla autumnalis deletions accounted for over 90% of spontaneous change observed in tip mitoses, interchanges and particularly inversions identified by centric shifts being very unusual. Symmetrical pericentric and most paracentric inversions in this species cannot be detected and all two-break events (inversions and interchanges) are expected to be much less frequent than single break events (terminal deletions).

Spontaneous breakage, as an event of low frequency, is distributed amongst cells as the terms of a Poisson distribution and in Scilla autumnalis fragment number is distributed as a Poisson. Similarly in sperm of

Tetranychus urticae the frequency of chromosome breaks fitted a Poisson distribution (Tempelaar, 1979) and this is also the case in Trillium grandiflorum endosperm (Rutishauser, 1956).

The overall level of spontaneous structural change in Scilla autumnalis root tips increased with ploidy level from 0.45 changes per 100 diploid cells (Table 5.1) to 1.86 for tetraploid and 4.72 for hexaploids. This is not simply a feature of increased chromosome number since the number of aberrations per genome increases from 0.45 per genome per 100 cells in diploids to 0.93 in tetraploids and 1.57 in hexaploids (Table 5.3). Thus for each additional two genomes there is a two-fold increase in the level of spontaneous structural change per genome.

Geok-Yong and Dunn (1977) similarly observed that the frequency of mitotic irregularities in root tip cells of Bromus inermis increases between tetraploids and hexaploids although surprisingly a commercial octoploid variety was the most stable.

The hybrid nature of the autoallohexaploid S. autumnalis may in part account for the higher level of aberration in this race since hybrids are known to have an increased frequency of breakage (Rutishauser and LaCour, 1956; Heneen, 1963). In this respect it would be instructive to compare aberration frequency in the allotetraploid AABB with that in the autotetraploid BBBB race of S. autumnalis described in this thesis.

The levels of spontaneous aberrations in Scilla autumnalis reported here correspond well with those in some other species: Najas marina root-tip mitoses 0.56% (Viinikka et al., 1978), Tradescantia pollen grain mitoses 0.11% (Giles, 1940), Trillium grandiflorum endosperm 1.06% (Rutishauser, 1956). Knuutila et al. (1977) found spontaneous breakages in human bone marrow cells at a frequency of 0.9% whilst Aula and Koskull (1976) cite 1.9% for human lymphocytes. In some systems, however, higher levels of aberrations have been observed. In roots of Allium cepa Cebrat (1977) recorded frequencies of 2.1-8.9% which he

ascribed to a parasitic origin. Aberration frequencies of up to a remarkable 21.2% were observed in Clivia miniata (Kato, 1960), the higher values correlating with increased seasonal temperature.

In Scilla autumnalis spontaneous chromosome breaks and also interchanges were distributed equally amongst the chromosomes. In Trillium grandiflorum (Rutishauser, 1956) by contrast, the breaks were not distributed equally between the chromosomes although the various regions of a chromosome were in general randomly affected. An exception was the heterochromatic region of chromosome D which was favoured for breakage. This tendency for chromosomal breaks to occur near to H-segments has also been shown by Barton (1954) in tomato.

Studies of radiation-induced spontaneous chromosome breakage show that organisms are heterogeneous with respect to the distribution of breaks between chromosomes. Kaufman (1946, 1954) claims that breakage in Drosophila spermatozoa (leading to viable rearrangement) is distributed randomly. In somatic cells of Vicia faba (Read, 1959) the distribution of aberrations correlates with the metaphase chromosome length. However, relative chromosome length may not necessarily be a true reflection of the length of the chromosome at the time of breakage, since heterochromatic regions may undergo differential contraction during the course of cell division (Smith, 1965). Studies on other organisms, particularly insects, have revealed considerable deviation from non-randomness (White, 1935).

In Scilla autumnalis there appears to be two distinct classes of chromosome breakage. Firstly, there are cells which have between 1 and 6 fragments or a small number of other chromosomal aberrations such as interchanges and deletions. Secondly, there are cells in which the chromosomes have undergone extreme fragmentation giving a vast number of fragments. The second type are characterised by fragments of a great

range of sizes with few of no chromosomes unaffected. Such extremely fragmented cells appear to be qualitatively different from cells which have undergone more moderate breakage, and no intermediates are found. This might suggest that the two levels of fragmentation are independently determined. There may, however, be a threshold below which only moderate fragmentation occurs but above which the overall control of the cell breaks down leading to extreme fragmentation. Cells brought prematurely into division during S-phase show chromosome pulverisation of this type (Johnson & Rao, 1971) and this may suggest a connection with defective synthesis. A similar case of extreme fragmentation has been reported in mitotic cells of triploid Tradescantia virginiana (Darlington and Upcott, 1941). Extreme fragmentation has also been noted in meiotic cells of Lilium longiflorum (Emsweller and Brearly, 1943), Leontodon hispidus (Bergman, 1935), Kinugasa japonica (Haga, 1937), Scilla sibirica (Rees, 1952) and Paeonia californica (Walters, 1956). In S. autumnalis too, extreme fragmentation has been recorded in meiotic cells of two hexaploid plants (Plate 5.1 dd,ee).

The underlying mechanisms leading to spontaneous breakage are largely unknown. In general, the process of chromosomal aberration begins with a lesion at the level of the DNA. Some of these lesions are transformed into visible chromosomal aberrations by misreplication or misrepair mechanisms (Evans and Scott, 1969; Kihlman et al., 1978). Agents which promote breakage may be physical (such as irradiation) chemical (alkylating agents) or biological. Viruses have been implicated as biological agents which can cause extensive chromosome breakage (Knuutila et al., 1977; Cebrat, 1977). It has also been proposed that automutagenic substances may accumulate as normal products of metabolism (Marquardt, 1952).

At meiosis chromosome breakage during chiasma formation may result in U-type reunions rather than the normal X-type exchange (Jones, 1968). Although crossing-over does occur at mitosis sister-chromatid exchange is more frequent (Taylor, 1958; Kihlman and Kronberg, 1975; Geard, 1976). It is possible that U-type reunion may occur during somatic sister-chromatid exchange as in the formation of chiasmata (Viinikka, 1977). The consequences of U-type exchange then will be the production of bridges and fragments at mitotic anaphase. Breakage of such dicentric chromatid bridges will give chromosomes with apparent duplications and deletions. This then may be a potent generator of some of the somatic changes detected here in polyploid races of Scilla autumnalis.

# CHAPTER SIX

## POLYMORPHIC STRUCTURAL VARIATION

Details of the nature and frequency of polymorphic variants are given in Table 6.1.

### I. Diploid populations

#### Inversion polymorphisms

Inversion 1-3

Inv 1-3, a pericentric inversion in chromosome 1, was present as two heterozygotes in the Mt. Pantokrator population (Fig. 6.1, Plate 6.2d). This inversion shifted the centromere to a more media location resulting in an arm ratio of 1:1.95 (Fig. 5.2). The frequency of the variant chromosome was 0.017.

#### Duplication polymorphisms

Duplication 1-5

Dup 1-5, a chromosome 1 duplication, increases the length of the short arm by 3.7 μm (155%) giving a large metacentric chromosome (Fig. 5.3; Plate 6.3g,h). This novel metacentric may be an isochromosome.

The duplication chromosome reaches polymorphic proportions in the Mt. Pantokrator population, Corfu (Fig. 6.3). Six heterozygotes but no homozygotes were found in 30 plants, an overall frequency of 0.05 (Plate 6.3g). The morph frequencies appear to be in Hardy-Weinberg equilibrium though the numbers of plants are too small to test statistically. Although the adjacent diploid population MS contained no Dup 1-5 chromosomes remarkably a single plant in the tetraploid population to the west of MS contained a chromosome of identical

Table 6.1 Polymorphic structural variation in S. autumnalis - details of the rearrangements and the numbers of plants. The chromosome arm affected is shown as short (s) or long (l). Inversions are pericentric (peri) or paracentric (para). Figures in parentheses denote the expected numbers of plants on the basis of a Hardy-Weinberg equilibrium, $\chi^2$ values on the expected and observed numbers are all non-significant (P > 0.05).

| Polymorphism | Arm affected | Change in length (μm) | Arm ratio | Population | Number of plants | | | | | Chromosome frequency |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. of variant chromosomes | | | | Total | |
| | | | | | 0 | 1 | 2 | 3 | | |
| DELETION | | | | | | | | | | |
| Del 5-1 | 1 | 2.5 | 1:1.00 | ASJ (4x) | 30 (29.1) | 1 (2.8) | 1 (0.1) | - | 32 | 0.023 |
| INVERSION | | | | | | | | | | |
| Inv 1-1 | (peri) | - | 1:1.95 | PDA (4x) | 27 (26.2) | 7 (8.7) | 2 (1.1) | - | 36 | 0.076 |
| Inv 1-2 | (peri) | - | 1:2.11 | CB (4x) | 28 (28.1) | 3 (2.8) | - | - | 31 | 0.024 |
| | | | | FC (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| | | | | FR (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| Inv 1-3 | (peri) | - | 1:2.05 | PK (2x) | 28 (28.1) | 2 (1.9) | - | - | 30 | 0.017 |
| Inv 1-4 | (peri) | | 1:1.29 | VP (4x) | 29 (29.1) | 2 (1.9) | - | - | 31 | 0.016 |
| | | | | FR (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| Inv 3-1 | (peri) | - | 1:2.64 | LE (6x) | 11 (11.2) | 11 (11.5) | 6 (4.5) | - | 28 | 0.205 |
| | | | | LC (6x) | 10 (12.2) | 15 (11.3) | 3 (3.9) | - | 28 | 0.188 |
| | | | | SMP (6x) | 15 (14.8) | 11 (12) | 5 (3.7) | - | 31 | 0.169 |
| | | | | CT (6x) | 21 (20.4) | 7 (8.3) | 2 (1.3) | - | 30 | 0.092 |
| | | | | RT (6x) | 29 (29.4) | 6 (5.3) | - | - | 35 | 0.043 |
| | | | | LL (6x) | 26 (26.1) | 3 (2.8) | - | - | 29 | 0.026 |
| | | | | PP (4x) | 13 (14.5) | 15 (12.7) | 4 (4.2) | - | 32 | 0.180 |
| | | | | MP (4x) | 16 (14.5) | 7 (10.4) | 5 (2.8) | - | 28 | 0.152 |
| | | | | FC (4x) | 15 (15.7) | 12 (11.1) | 3 (2.9) | - | 30 | 0.150 |
| | | | | BH (4x) | 29 (28.2) | 17 (19.3) | 7 (5) | - | 53 | 0.146 |
| | | | | LQP (4x) | 19 (17.7) | 10 (11.9) | 3 (3.0) | 1 (0.3) | 33 | 0.144 |
| | | | | C+C (4x) | 18 (18.6) | 13 (12.1) | 3 (2.9) | - | 34 | 0.140 |
| | | | | BN (4x) | 20 (18.8) | 8 (10.7) | 4 (2.3) | - | 32 | 0.125 |
| | | | | GN (4x) | 20 (20.4) | 9 (8.2) | 1 (1.2) | - | 30 | 0.092 |
| | | | | PH (4x) | 20 (20.4) | 9 (8.2) | 1 (1.2 | - | 30 | 0.092 |
| | | | | SG (4x) | 110 (110) | 36 (36.3) | 5 (4.5) | - | 151 | 0.076 |
| | | | | CCF (4x) | 21 (22) | 9 (7.1) | - | - | 30 | 0.075 |
| | | | | PDT (4x) | 25 (22.7) | 3 (7.9) | 2 (0.7) | - | 30 | 0.067 |
| | | | | CB (4x) | 25 (25.6) | 5 (5.9) | 1 (0.5) | - | 31 | 0.057 |
| | | | | ASJ (4x) | 27 (26.4) | 4 (5.2) | 1 (0.4) | - | 32 | 0.050 |
| | | | | LH (4x) | 22 (22.3) | 5 (4.5) | - | - | 27 | 0.046 |
| | | | | PAL (4x) | 18 (18.3) | 4 (3.5) | - | - | 22 | 0.046 |
| | | | | BSM (4x) | 26 (26.3) | 5 (4.4) | - | - | 31 | 0.040 |
| | | | | PDG (4x) | 23 (23.1) | 3 (2.7) | - | - | 26 | 0.029 |
| | | | | MG (4x) | 27 (27.1) | 3 (2.8) | - | - | 30 | 0.025 |
| | | | | PDP (4x) | 27 (27.1) | 3 (2.8) | - | - | 30 | 0.025 |
| | | | | ADC (4x) | 26 (26.1) | 2 (1.9) | - | - | 28 | 0.018 |
| | | | | LV (4x) | 27 (27.1) | 2 (1.9) | - | - | 29 | 0.017 |
| | | | | SSL (4x) | 29 (29.1) | 2 (1.9) | - | - | 31 | 0.016 |
| | | | | PDA (4x) | 34 (34) | 2 (1.9) | - | - | 36 | 0.014 |
| | | | | PSG (4x) | 23 (23) | 1 (1) | - | - | 24 | 0.010 |
| | | | | HC (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| | | | | VP (4x) | 30 (30) | 1 (1) | - | - | 31 | 0.008 |
| | | | | IOW (4x) | 35 (35) | 1 (1) | - | - | 36 | 0.007 |

/Continued

Table 6.1 continued

| Polymorphism | Arm affected | Change in length (μm) | Arm ratio | Population | Number of plants | | | | | Chromosome frequency |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. of variant chromosomes | | | | Total | |
| | | | | | 0 | 1 | 2 | 3 | | |
| Inv 3-2 | (peri) | - | 1:1.42 | PDA (4x) | 33 (33.1) | 3 (2.8) | - | - | 36 | 0.021 |
| Inv 3-3 | (para) | - | 1:1.75 | LV (4x) | 27 (27.1) | 2 (1.9) | - | - | 29 | 0.017 |
| Inv 3-4 | (peri) | - | 1:2.05 | GP (4x) | 27 (27.1) | 2 (1.9) | - | - | 29 | 0.017 |
| Inv 3-5 | (peri) | - | 1:1.21 | CB (4x) | 29 (29.1) | 2 (1.9) | - | - | 31 | 0.016 |
| Inv 3-6 | (para) | - | 1:1.73 | AS (4x) | 17 (16.8) | 5 (5.5) | 1 (0.7) | - | 23 | 0.076 |
| | | | | KV (4x) | 20 (19.3) | 3 (4.3) | 1 (0.3) | - | 24 | 0.052 |
| Inv 3-7 | (peri) | - | 1:1.43 | AS (4x) | 21 (21.9) | 2 (1.9) | - | - | 23 | 0.022 |
| Inv 3-8 | (para) | - | 1:1.73 | ASJ (4x) | 28 (27.2) | 3 (4.4) | 1 (0.3) | - | 32 | 0.039 |
| Inv 3-9 | (pari) | - | 1:2.50 | LL (6x) | 17 (17.3) | 10 (9.5) | 2 (2.0) | - | 29 | 0.121 |
| | | | | LC (6x) | 27 (27) | 1 (1) | - | - | 28 | 0.009 |
| Inv 3-10 | (peri) | - | 1:1.18 | IOW (4x) | 34 (34) | 2 (1.9) | - | - | 36 | 0.014 |
| Inv 3-11 | (peri) | - | 1:2.45 | GR (4x) | 28 (28.2) | 4 (3.6) | - | - | 32 | 0.031 |
| Inv 3-12 | (peri) | - | 1:1.21 | SG (4x) | 146 (145) | 3 (5.8) | 2 (0.1) | - | 151 | 0.012 |
| Inv 5-1 | (peri) | - | 1:1.33 | BSM (4x) | 28 (28.1) | 3 (2.8) | - | - | 31 | 0.024 |
| Inv 6-1 | (peri) | - | 1:3.71 | BSM (4x) | 8 (9.4) | 14 (13.1) | 9 (6.8) | - | 31 | 0.258 |
| | | | | LM (4x) | 2 (2.7) | 4 (2.9) | 1 (1.2) | - | 7 | 0.214 |
| | | | | GN (4x) | 12 (13.9) | 15 (11.8) | 3 (3.8) | - | 30 | 0.175 |
| | | | | MG (4x) | 14 (15.7) | 14 (11.1) | 2 (2.9) | - | 30 | 0.150 |
| | | | | FR (4x) | 17 (18.3) | 12 (9.6) | 1 (1.9) | - | 30 | 0.117 |
| | | | | CB (4x) | 26 (26.3) | 5 (4.4) | - | - | 31 | 0.040 |
| | | | | VP (4x) | 27 (26.3) | 3 (4.4) | 1 (0.3) | - | 31 | 0.040 |
| | | | | LH (4x) | 23 (23.2) | 4 (3.6) | - | - | 27 | 0.037 |
| | | | | LV (4x) | 26 (26.1) | 3 (2.8) | - | - | 29 | 0.026 |
| | | | | PSG (4x) | 23 (23) | 1 (1) | - | - | 24 | 0.010 |
| | | | | PDT (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| | | | | SG (4x) | 150 (150) | 1 (1) | - | - | 151 | 0.002 |
| Inv 6-2 | (peri) | - | 1:1.00 | SG (4x) | 148 (148) | 3 (3) | - | - | 151 | 0.005 |
| Inv 7-1 | (peri) | - | 1:2.61 | PDT (4x) | 28 (28) | 2 (1.9) | - | - | 30 | 0.017 |
| Inv 7-2 | (peri) | - | 1:1.78 | FC (4x) | 27 (27.1) | 7 (2.8) | - | - | 30 | 0.025 |
| Inv 7-3 | (peri) | - | 1:2.13 | RT (6x) | 33 (33) | 2 (1.9) | - | - | 35 | 0.014 |
| DUPLICATION | | | | | | | | | | |
| Dup 1-1 | s | 1.2 | 1:1.69 | BH (4x) | 25 (26.9) | 23 (19.9) | 5 (5.5) | - | 53 | 0.156 |
| | | | | LQP (4x) | 20 (20.1) | 10 (9.9) | 2 (1.8) | - | 32 | 0.109 |
| | | | | IC (4x) | 26 (24.8) | 5 (7.3) | 2 (0.8) | - | 33 | 0.068 |
| | | | | PP (4x) | 27 (27.3) | 5 (4.4) | - | - | 32 | 0.039 |
| Dup 1-2 | l | 1.3 | 1:3.07 | PDP (4x) | 24 (23.6) | 5 (5.8) | 1 (0.5) | - | 30 | 0.058 |
| | | | | LV (4x) | 26 (25.2) | 2 (3.6) | 1 (0.2) | - | 29 | 0.035 |
| | | | | PDT (4x) | 27 (26.2) | 2 (3.6) | 1 (0.2) | - | 30 | 0.033 |
| | | | | SSL (4x) | 29 (29.1) | 2 (1.9) | - | - | 31 | 0.016 |
| | | | | PSG (4x) | 23 (23) | 1 (1) | - | - | 24 | 0.010 |
| | | | | ADC (4x) | 27 (27) | 1 (1) | - | - | 28 | 0.009 |
| | | | | BSM (4x) | 30 (30) | 1 (1) | - | - | 31 | 0.008 |
| Dup 1-3 | l | 1.5 | 1:3.18 | VP (4x) | 8 (10.2) | 16 (13.1) | 7 (6.3) | - | 31 | 0.242 |
| | | | | CB (4x) | 29 (29) | 2 (1.9) | - | - | 31 | 0.016 |
| | | | | FR (4x) | 29 (29) | 1 (1) | - | - | 30 | 0.008 |
| Dup 1-4 | s | 0.6 | 1:2.05 | ADC (4x) | 26 (26.1) | 2 (1.9) | - | - | 28 | 0.018 |
| Dup 1-5 | s | 3.7 | 1:1.00 | PK (2x) | 24 (24.4) | 6 (5.1) | - | - | 30 | 0.050 |
| | | | | AS (4x) | 22 (22) | 1 (1) | - | - | 23 | 0.011 |

/Continued

Table 6.1 continued

| Polymorphism | Arm affected | Change in length (µm) | Arm ratio | Population | Number of plants: No. of variant chromosomes 0 | 1 | 2 | 3 | Total | Chromosome frequency |
|---|---|---|---|---|---|---|---|---|---|---|
| Dup 1-6 | s | 0.6 | 1:2.02 | MS (2x) | 17 (17.9) | 10 (8.1) | - | - | 27 | 0.185 |
| | | | | LK (2x) | 20 (20.2) | 4 (3.7) | - | - | 24 | 0.083 |
| | | | | AS (4x) | 21 (21.1) | 2 (1.9) | - | - | 23 | 0.022 |
| Dup 2-1 | s | 0.6 | 1:1.43 | PK (2x) | 22 (21.7) | 7 (7.7) | 1 (0.7) | - | 30 | 0.150 |
| Dup 3-1 | 1 | 0.7 | 1:2.02 | SSL (4x) | 27 (27.2) | 4 (3.6) | - | - | 31 | 0.032 |
| | | | | PDP (4x) | 28 (28) | 2 (1.9) | - | - | 30 | 0.017 |
| | | | | PAL (4x) | 21 (21) | 1 (1) | - | - | 22 | 0.014 |
| Dup 3-2 | 1 | 0.3 | 1:1.85 | SG (4x) | 147 (147) | 4 (3.9) | - | - | 151 | 0.007 |
| Dup 3-4 | 1 | 0.4 | 1:1.90 | VP (4x) | 26 (26.3) | 5 (4.4) | - | - | 31 | 0.040 |
| Dup 3-5 | 1 | 1.0 | 1:2.17 | KV (4x) | 20 (19.3) | 2 (3.5) | 1 (0.2) | - | 23 | 0.044 |
| Dup 3-6 | s | 0.6 | 1:1.38 | KV (4x) | 21 (21.1) | 2 (1.9) | - | - | 23 | 0.022 |
| Dup 3-7 | 1 | 0.8 | 1:2.06 | LK (2x) | 23 (21.6) | 2 (1.9) | - | - | 24 | 0.043 |
| | | | | MS (2x) | 26 (26) | 1 (1) | - | - | 27 | 0.019 |
| Dup 3-8 | 1 | 0.7 | 1:2.02 | RT (6x) | 33 (33) | 2 (1.9) | - | - | 35 | 0.014 |
| Dup 3-9 | 1 | 0.9 | 1:2.09 | PDG (4x) | 24 (23.1) | 1 (2.7) | 1 (0.1) | - | 26 | 0.029 |
| Dup 3-10 | 1 | 0.7 | 1:3.05 | PDG (4x) | 22 (22) | 4 (3.6) | - | - | 26 | 0.039 |
| | 1 | 1.9 | 1:3.48 | LV (4x) | 27 (27.1) | 2 (1.9) | - | - | 29 | 0.017 |
| | | | | CB (4x) | 29 (29.1) | 2 (1.9) | - | - | 31 | 0.016 |
| | | | | PAL (4x) | 21 (21) | 1 (1) | - | - | 21 | 0.011 |
| | | | | LH (4x) | 26 (26) | 1 (1) | - | - | 27 | 0.009 |
| | | | | ASJ (4x) | 31 (31) | 1 (1) | - | - | 32 | 0.008 |
| | | | | PDA (4x) | 35 (35) | 1 (1) | - | - | 36 | 0.007 |
| Dup 5-1 | 1 | 2.3 | 1:3.39 | SG (4x) | 148 (148) | 3 (3) | - | - | 151 | 0.005 |
| Dup 5-2 | 1 | 2.9 | 1:3.66 | AS (4x) | 16 (14.5) | 4 (7.1) | 3 (1.3) | - | 23 | 0.109 |
| | | | | KV (4x) | 20 (18.4) | 1 (4.2) | 2 (0.4) | - | 23 | 0.054 |
| Dup 6-1 | s | 1.8 | 1:1.00 | AS (4x) | 19 (18.4) | 3 (4) | 1 (0.4) | - | 23 | 0.054 |
| | | | | KV (4x) | 20 (20) | 3 (2.7) | - | - | 23 | 0.033 |
| Dup 7-1 | - | 1.4 | 1:1.56 | AS (4x) | 20 (20) | 3 (2.7) | - | - | 23 | 0.033 |
| | | | | KV (4x) | 20 (20) | 3 (2.7) | - | - | 23 | 0.033 |

Fig. 6.1 Inversion polymorphisms present in single populations. The frequencies of particular inversion chromosomes are indicated on pie diagrams. The island of Corfu is inset. ■ = diploid, ▲ = autotetraploid, ● = autoallohexaploid.

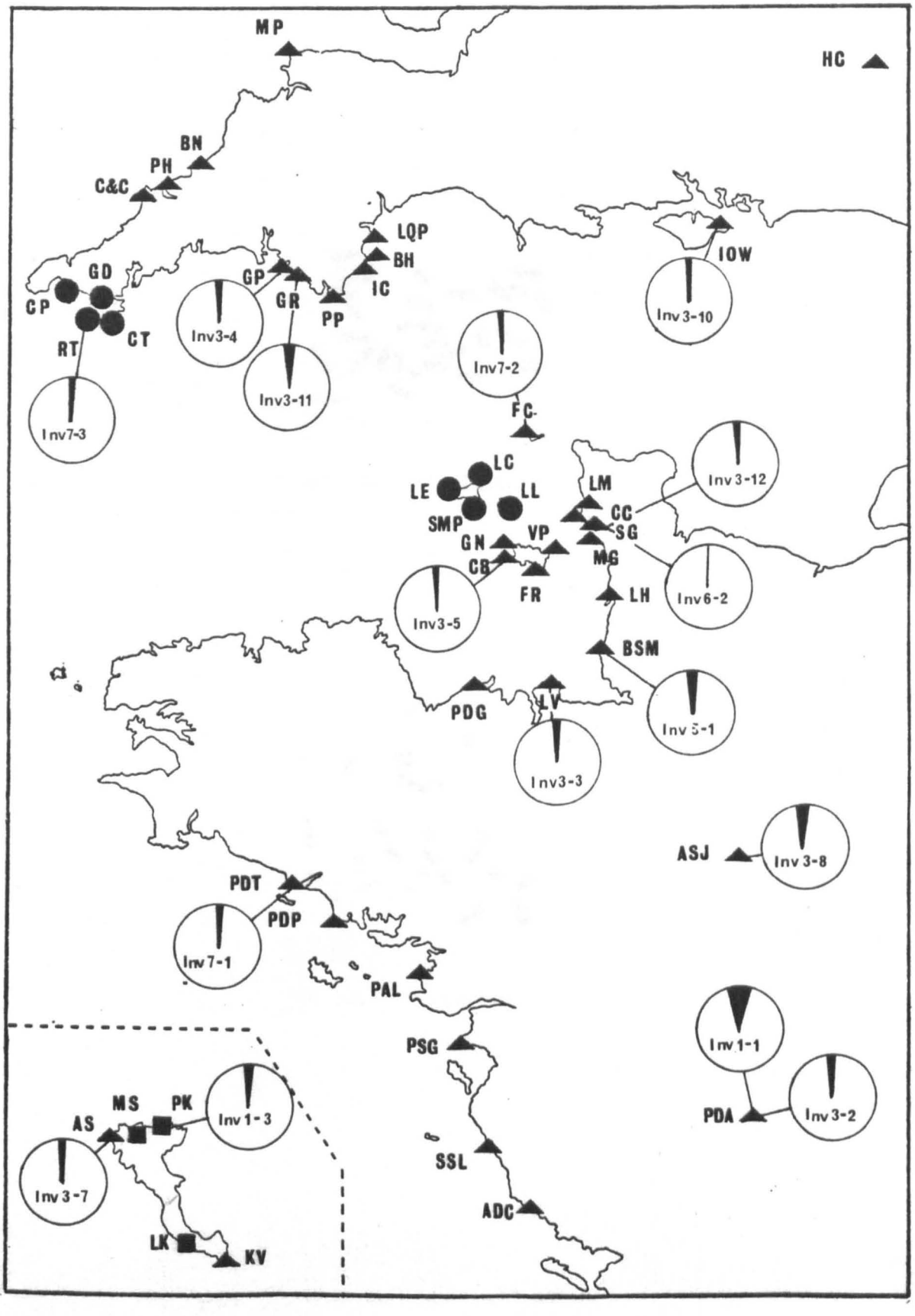


Plate 6.1 A deletion polymorphism (Del 5-1)

(a) Del 5-1 simplex

(b) Del 5-1 duplex

Plate 6.2 Polymorphic inversions

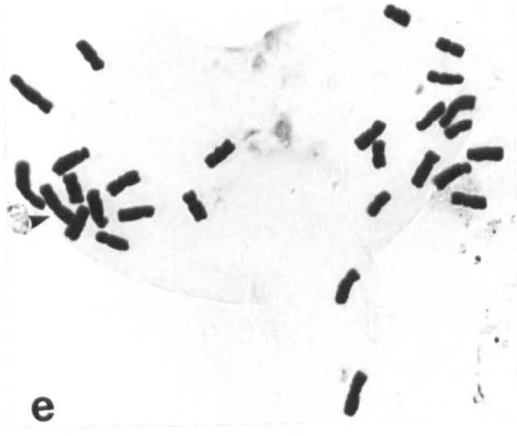
a Inv 1-1 simplex
b Inv 1-1 duplex
c Inv 1-2 simplex
d Inv 1-3 heterozygote
e Inv 1-4 simplex
f Inv 3-1 simplex (4x)
g Inv 3-1 duplex (4x)
h Inv 3-1 triplex (4x)
i Inv 3-1 simplex (6x)
j Inv 3-3 simplex
k Inv 3-5 simplex
l Inv 3-6 simplex
m Inv 3-6 duplex
n Inv 3-7 simplex
o Inv 3-8 simplex
p Inv 3-9 simplex
q Inv 3-10 simplex
r Inv 3-11 simplex
s Inv 3-12 simplex
t Inv 3-12 duplex
u Inv 5-1 simplex
v Inv 6-1 simplex
w Inv 6-1 duplex
x Inv 6-2 simplex
y Inv 7-1 simplex
z Inv 7-2 simplex


a


b


c


d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g


h


i


j

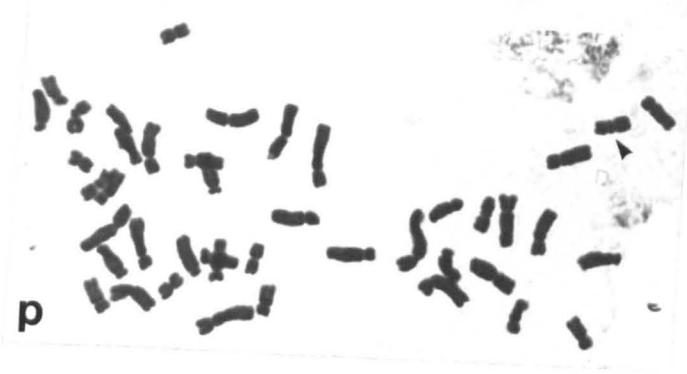

k


l


m


n


o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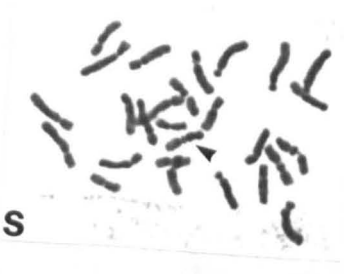
r


q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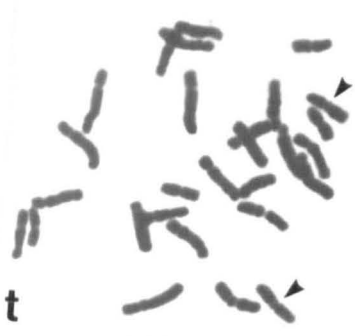
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u


v


w


x


y


z

Figure 6.2 Duplication polymorphisms present in single populations.

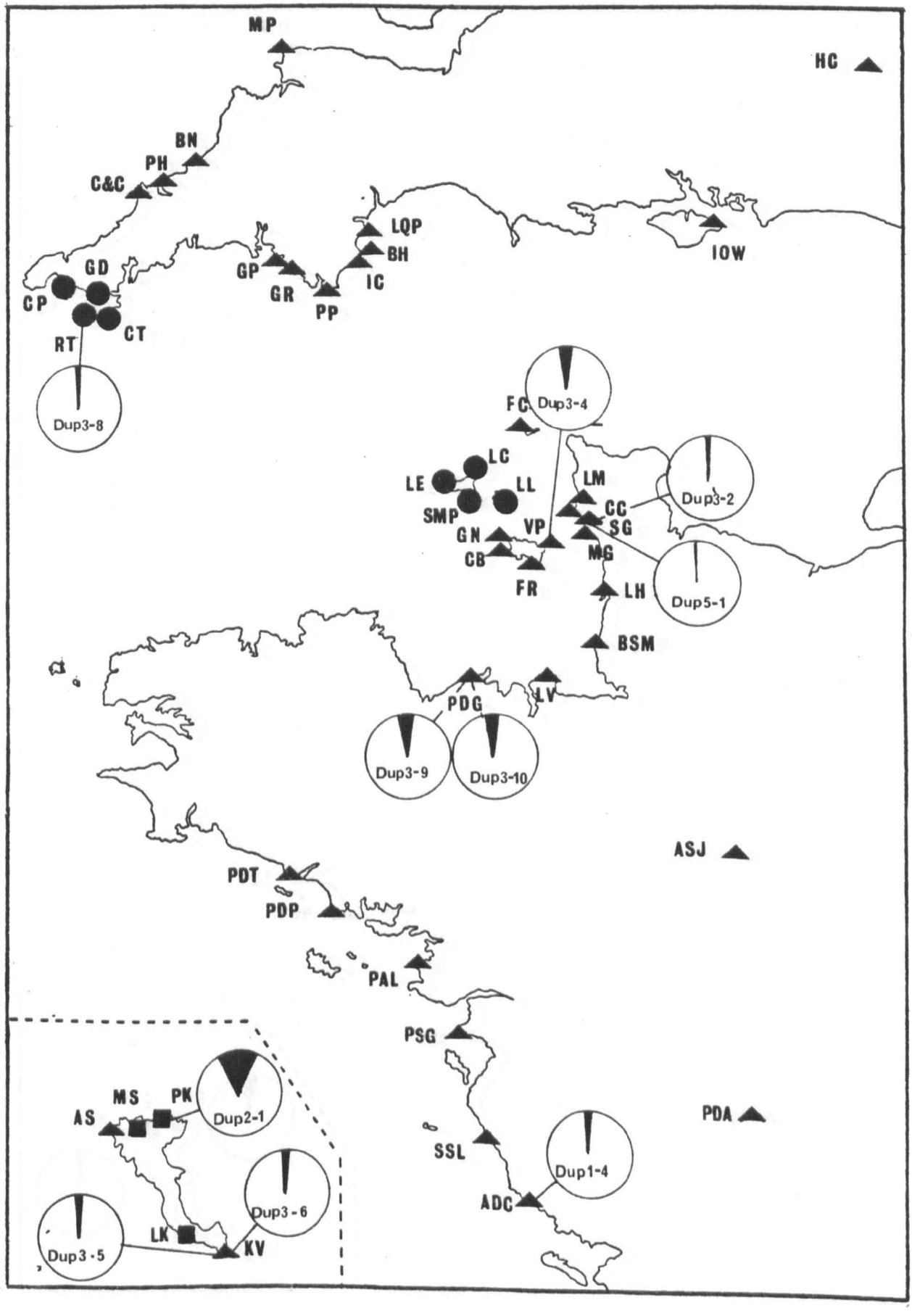


Figure 6.3 Distribution and frequency of Dup 1-5.

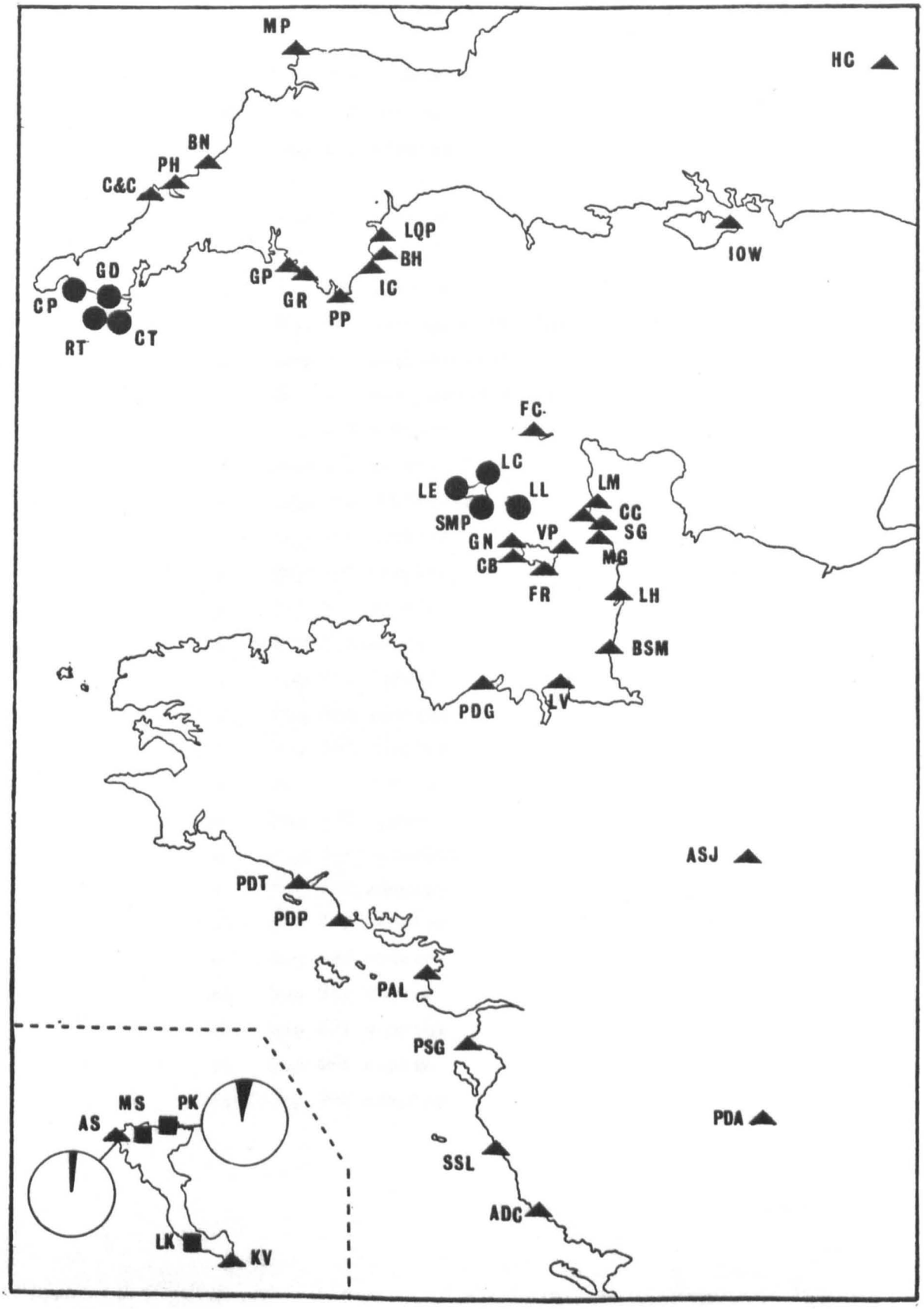
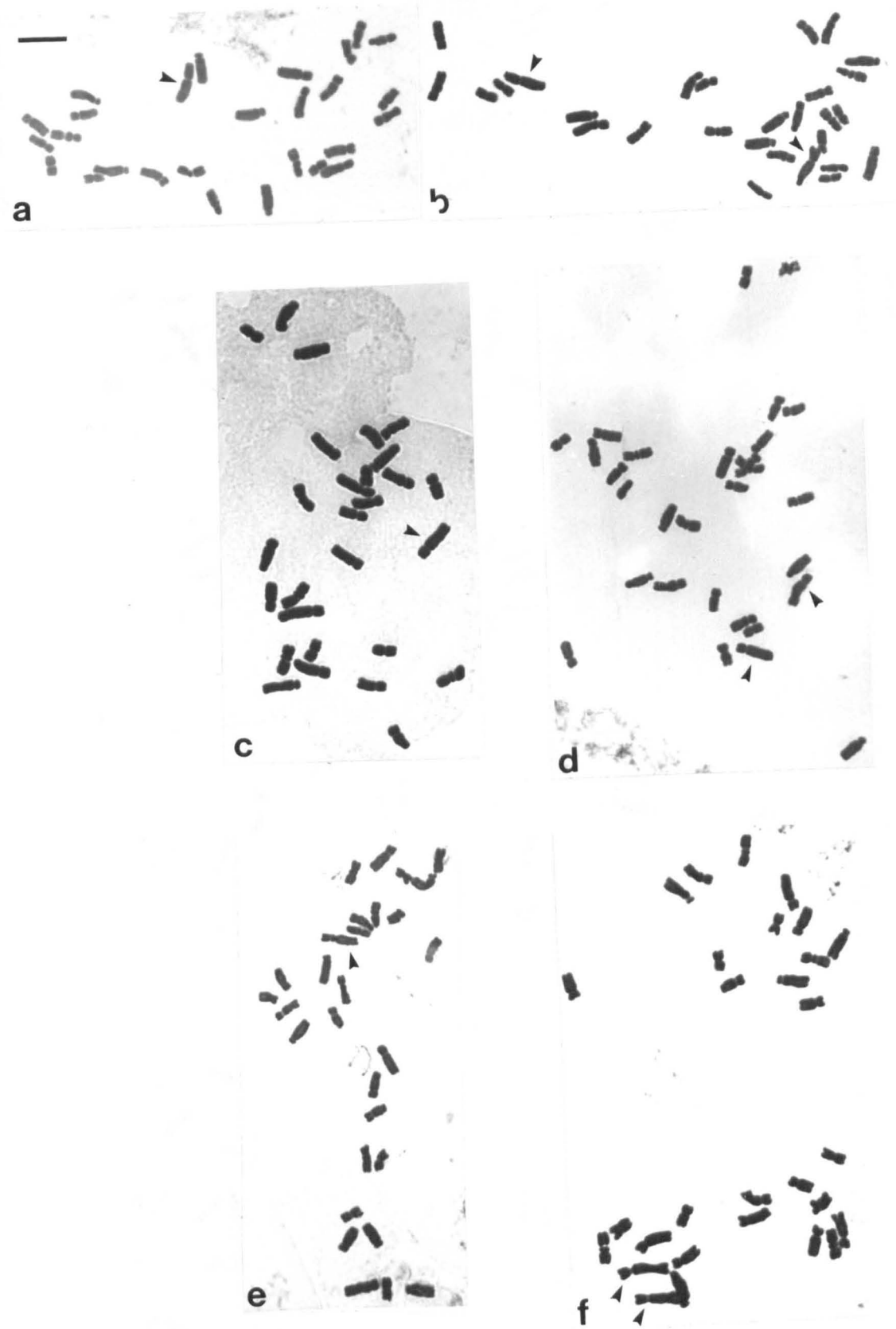
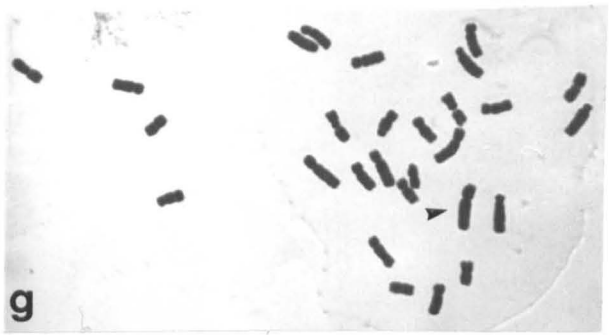


Plate 6.3 Polymorphic duplications

a Dup 1-1 simplex
b Dup 1-1 duplex
c Dup 1-2 simplex
d Dup 1-2 duplex
e Dup 1-3 simplex
f Dup 1-3 duplex
g Dup 1-4 simplex
h Dup 1-5 heterozygote (2x)
i Dup 1-5 simplex (4x)
j Dup 1-6 heterozygote (2x)
k Dup 1-6 simplex (4x)
l Dup 2-1 heterozygote
m Dup 2-1 homozygote
n Dup 3-1 simplex
o Dup 3-2 simplex
p Dup 3-4 simplex
q Dup 3-5 simplex
r Dup 3-5 duplex
s Dup 3-6 simplex
t Dup 3-7 simplex
u Dup 3-9 simplex
v Dup 3-9 duplex
w Dup 3-10 simplex
x Dup 4-1 simplex
y Dup 5-1 simplex
z Dup 5-2 simplex
aa Dup 5-2 duplex
bb Dup 6-1 simplex
cc Dup 6-1 duplex
dd Dup 7-1 simplex


a
b
c
d
e
f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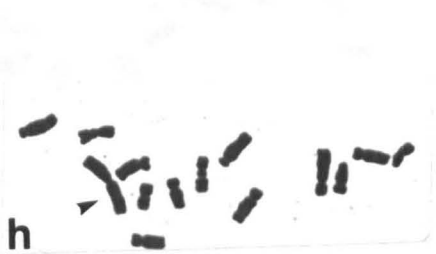
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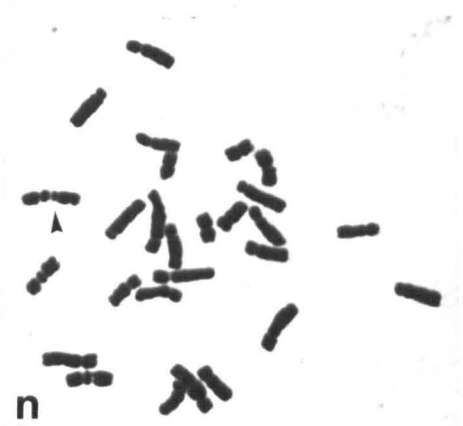
k


l


m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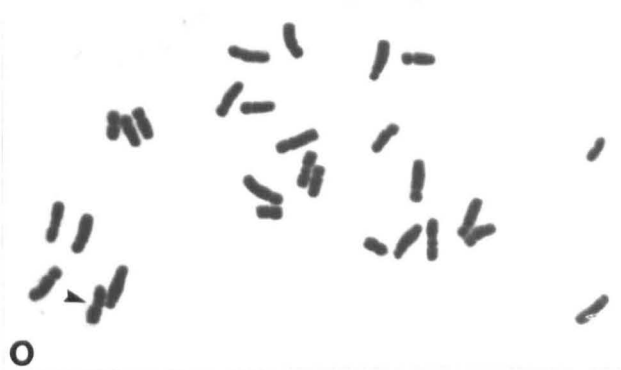
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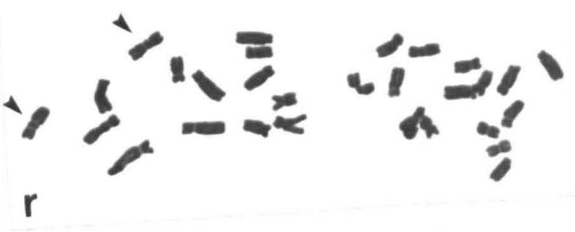
q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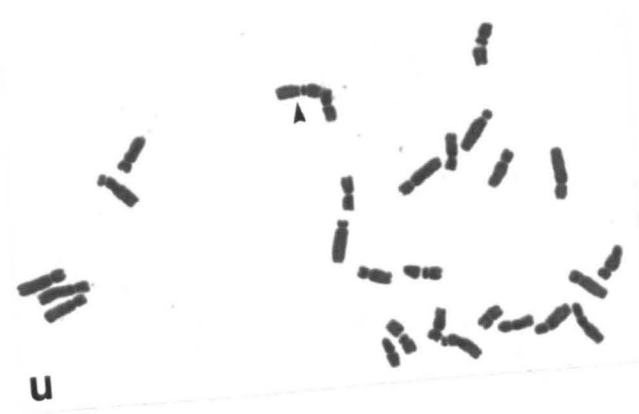
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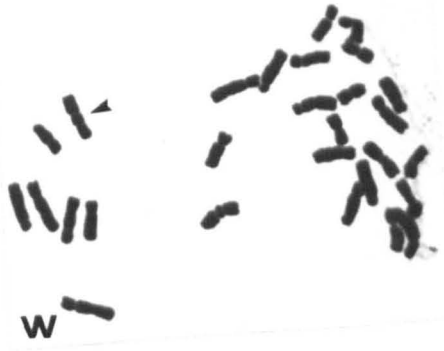
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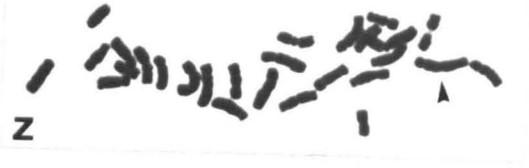
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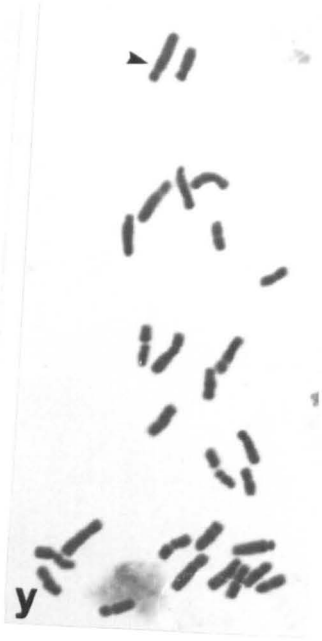
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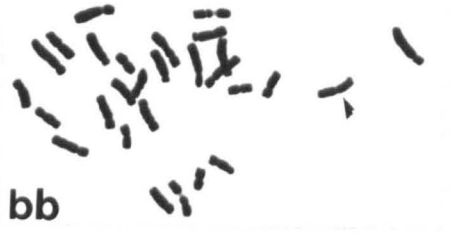
aa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dd

morphology to Dup 1-5. If this metacentric is Dup 1-5 then its origin may either predate the origin of the tetraploid or, more likely, have been transferred across the ploidy barrier by hybridisation and back-crossing. Mixed diploid/tetraploid populations have not yet been found. Meiotic analysis is required to establish the identity of Dup 1-5 in the two ploidy levels.

Duplication 1-6

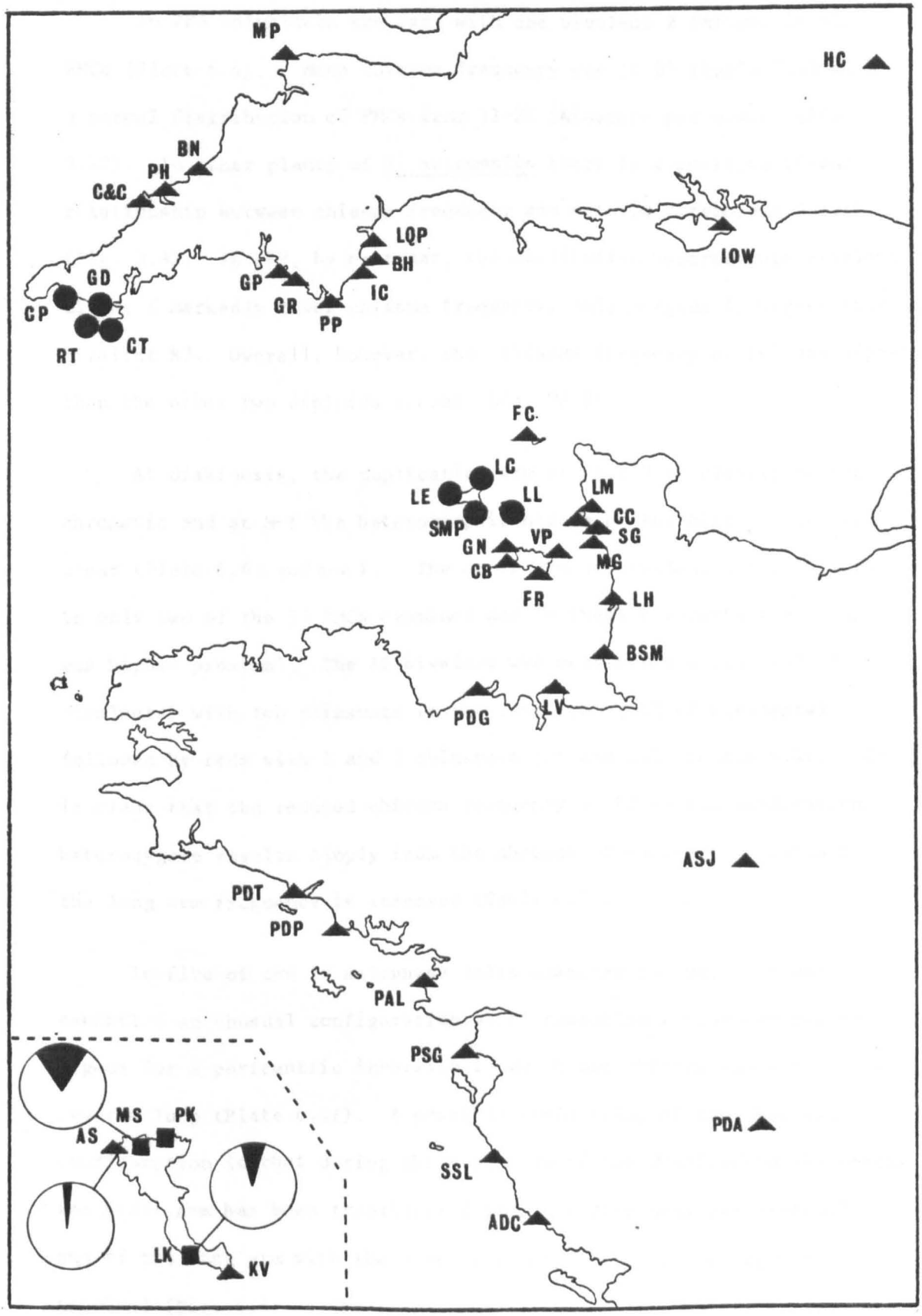
A chromosome referred to as Dup 1-6 was found in two diploid populations (Meseena and Lake Korillion) and one tetraploid (Aghios Stephanos) population located between them (Fig. 6.4). The duplication increased the length of the short arm of chromosome 1 by 0.6 μm (27%) (Plate 6.3j).

The frequency of the duplication chromosome was highest in the Meseena population (0.185) with 10 heterozygotes in 27 plants and lowest in the tetraploid population (0.023). The other diploid population, MS, was intermediate (0.083). As with Dup 1-5 meiotic analysis of diploid/tetraploid hybrids is required to establish similarity across ploidy level.

Duplication 2-1

This duplication increased the length of the short arm of chromosome 2 by 0.6 μm (53%) (Fig. 5.3). The polymorphism is restricted to the Mt. Pantokrator population where the frequency of the duplication chromosome was 0.15 (Fig. 6.2). Seven heterozygotes and one duplication homozygote were found, and the distribution corresponded to a Hardy-Weinberg equilibrium (Plate 6.3 l and m ). The duplicated chromosome arm is wider than the long arm or the short arm of the standard homologue in heterozygotes.

Figure 6.4 Distribution and frequency of Dup 1-6.



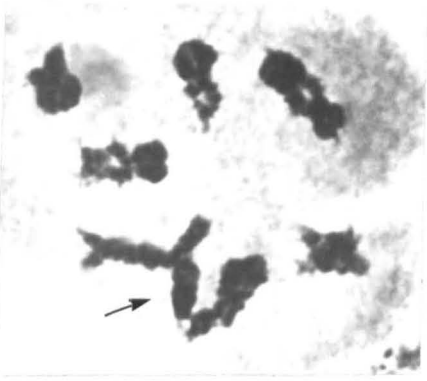
Meiosis in a Dup 2-1 heterozygote (PK 3)

In PK3 univalents are rare with one bivalent 2 failure in 53 PMCs (Plate 6.4). Mean chiasma frequency was 16.87 (Table 3.4) with a normal distribution of PMCs from 11-21 chiasmata per p.m.c. (Fig. 3.10). In other plants of S. autumnalis there is a positive linear relationship between chiasma frequency and mitotic chromosome length (Fig. 3.9). In PK3, by contrast, the duplication heterozygote bivalent B2 has a markedly lower chiasma frequency, only marginally higher than bivalent B7. Overall, however, the chiasma frequency of PK3 was higher than the other two diploids scored (LK3, MS18).

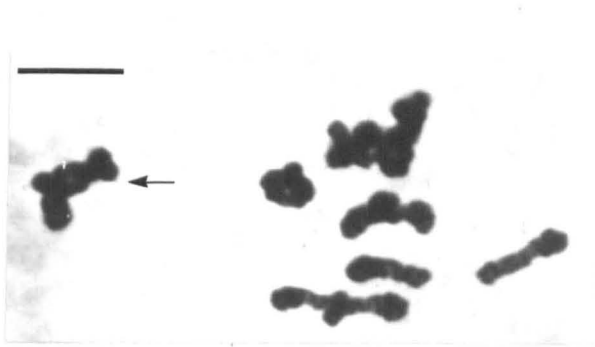
At diakinesis, the duplication arm of pair 2 is clearly heterochromatic and at M-I the heteromorphic nature of the bivalent is very clear (Plate 6.4a and b-e). The short arm of bivalent 2 was chiasmate in only two of the 53 PMCs examined and in these bivalents the chiasma was highly proximal. The B2 bivalent was most often a rod (85% of bivalents) with two chiasmata in the long arm (41% of bivalents) followed by rods with 1 and 3 chiasmata (21 and 23%) (Plate 6.4). It is clear that the reduced chiasma frequency of B2 in the duplication heterozygote results simply from the absence of short arm chiasmata; the long arm frequency is standard (Table 6.3).

In five of the 53 metaphase cells examined the pair 2 bivalent exhibited an unusual configuration which resembled a bivalent heterozygous for a pericentric inversion in which one chiasma was within the reverse loop (Plate 6.4f). A possible explanation of the observed configuration is that during the evolution of the duplication chromosome the short arm has been translocated to a position near the proximal end of the long arm with the heterochromatic duplication segment terminal (Fig. 6.5). The translocation cannot involve the entire

Plate 6.4 Meiosis in a Dup 2-1 heterozygote (PK 3). Duplication bivalent arrowed (Bivalent 2)



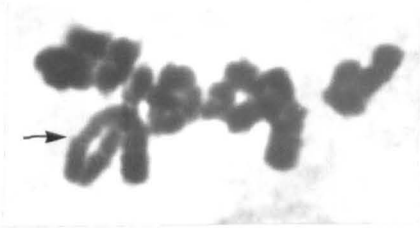
(a) Diakinesis 7II



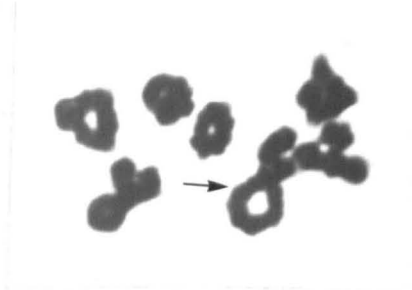
(b) M-I 6II + 2I Bivalent 2 ring

(c) M-I 7II Bivalent 2 rod (highly proximal chiasma)

(d) M-I 7II Bivalent 2 rod (interstitial chiasma)



(e) M-I 7II Bivalent 2 rod with two chiasmata, one terminal



(f) M-I 7II Bivalent 2 of unusual configuration (see text)

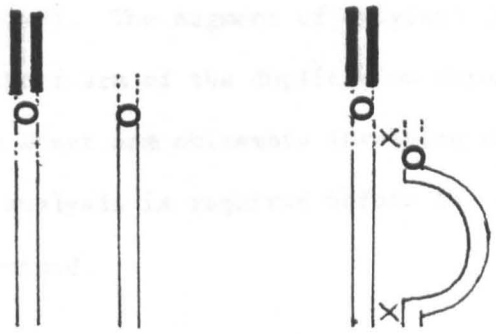
Table 6.2 Meiosis in a Dup 2-1 heterozygote (PK3): Chromosome pairing patterns

| Pairing Pattern | No. of chiasmata | No. of PMCs |
|---|---|---|
| Rod II | 1 | 11 (21%) |
| | 2 | 22 (41%) |
| | 3 | 12 (23%) |
| Ring II | 2 | 2 (4%) |
| Pair Is | 0 | 5 (9%) |
| Unknown | - | 1 (2%) |
| Total PMCs | | 53 |

Table 6.3 Meiosis in a Dup 2-1 heterozygote (PK3): B2 bivalent chiasma frequency

| Chromosome arm | B2 bivalent chiasma frequency | | |
|---|---|---|---|
| | MS18 | LK3 | PK3 |
| Short arm | 0.10 | 0.37 | 0.02 |
| Long arm | 1.95 | 2.16 | 1.98 |
| Total | 2.05 | 2.53 | 2.00 |

Figure 6.5 A proposed pairing scheme to account for observed M-I configurations in a duplication 2-1 heterozygote (PK3). See plate 6.4f.



Dup 2-1 Standard B2 Pairing

short arm since a single ring bivalent was observed which had a short arm chiasma close to the centromere.

The translocated short arm segment is not at the end of the long arm since bivalents were observed with terminal long arm chiasmata (Plate 6.4e). The segment of original (homologous) short arm remaining in the short arm of the duplication chromosome must be very short since only two short arm chiasmata involving this arm were observed. However, further analysis is required before the nature of this duplication can be determined.

Duplication 3-7

Dup 3-7 increased the length of the centromere - NO region of the long arm of chromosome 3 by 0.8 µm (21%) (Fig. 5.3). Two duplication heterozygotes were found in the Lake Korillion (Corfu) population, a frequency of 0.043 (Plate 6.3t). A second diploid population, Meseena, located to the north of LK contained a single individual which was heterozygous for a duplication similar to Dup 3-7 (Fig. 6.6). This population has also been classed as being polymorphic for this duplication although the level of 5% is not attained (3.7%) since Dup 3-7 is clearly widespread.

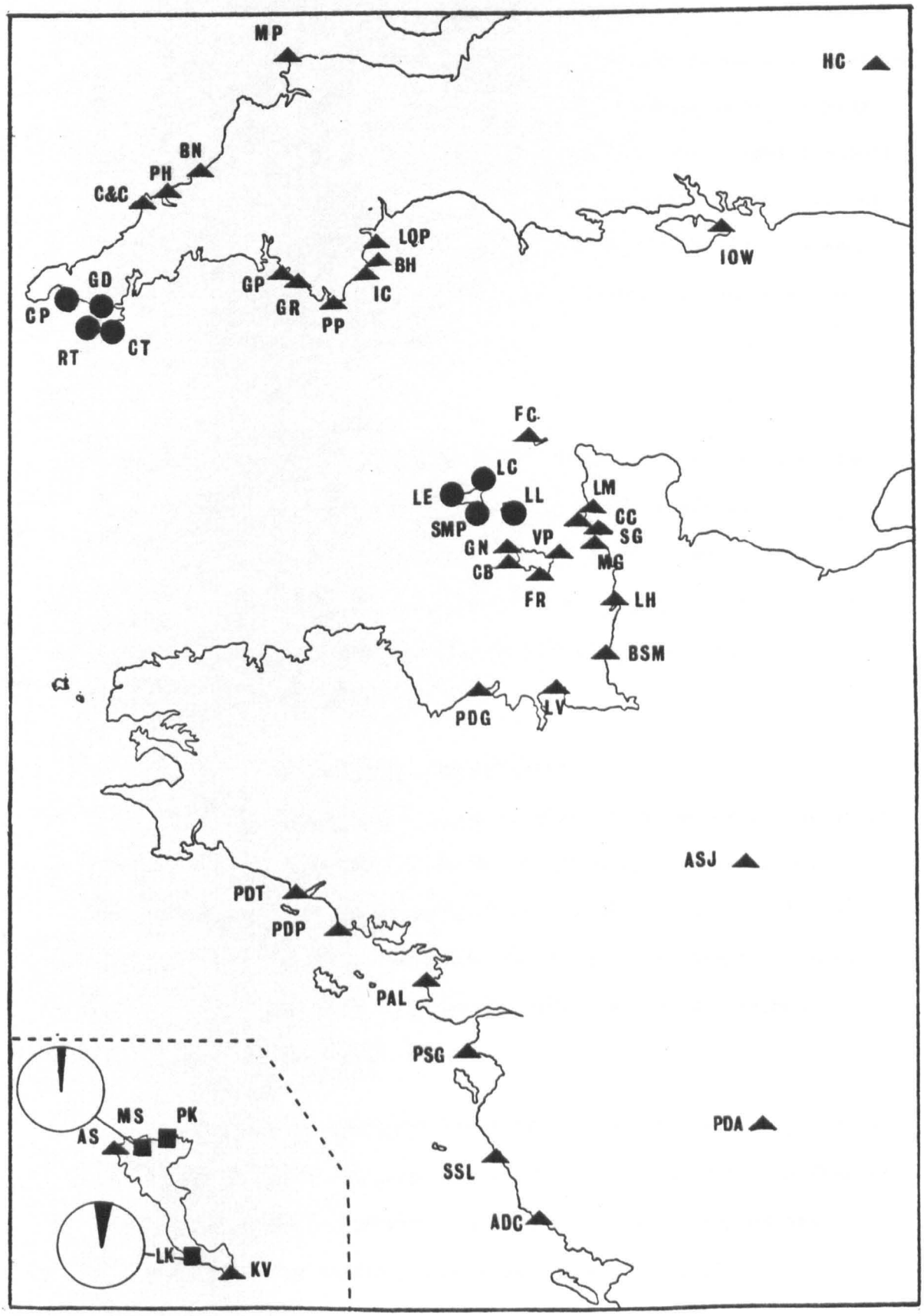
## II. Tetraploid Populations

### Inversion Polymorphisms

Inv 1-1

This inversion was confined to the Pont de l'Argenton population in France (Fig. 6.1). The pericentric inversion shifted the centromere of chromosome 1 to a more median position (Fig. 5.2). In the population of 36 plants, seven were simplex and two duplex for the inversion chromosome (frequency = 0.076; Plate 6.2 a and b ).

Figure 6.6 Distribution and frequency of Dup 3-7



Inv 1-2

Inv 1-2, a pericentric chromosome 1 inversion shifted the centromere to a more median position (Fig. 5.2). This chromosome was observed in three Channel Island populations (Fig. 6.7) although only reaching polymorphic proportions at Corbiere (Jersey) where three simplex plants were found (Plate 6.2c). Inversion chromosome frequency was 0.024. At Fort Corbelets (Alderney) and Fort Regent (Jersey) one plant in each population of 30 plants was found which was simplex for an inversion identical to that in the CB population.

Inv 1-4

Inv 1-4, a pericentric chromosome 1 inversion which shifted the centromere to a more median location giving an arm ratio of 1:1.29 was found at Vercl ut Point and Fort Regent, adjacent populations on Jersey (Fig. 5.2, Fig. 6.8). VP contained two simplex plants (inversion chromosome frequency 0.016) and FR a single plant which was also aneuploid with 2n = 31 (Plate 6.2e).

Inv 3-1, a widespread inversion polymorphism

In Inv 3-1, a pericentric inversion with break points in the short arm and the centromere - NO region of the long arm, the centromere is shifted to a more acrocentric position giving an arm ratio of 1:2.6 (Fig. 5.2). The inversion chromosome is conspicuous in mitotic metaphase cells since the short arm and the long arm proximal segment are equal in length (Plate 6.2d).

The inversion is extremely widespread occurring in six hexaploid and twenty-eight tetraploid populations throughout Britain, the Channel Islands and north-western France. Individuals simplex, duplex and triplex for the inversion have been found (Plate 6.2 d-i).

Figure 6.7 Distribution and frequency of Inv 1-2

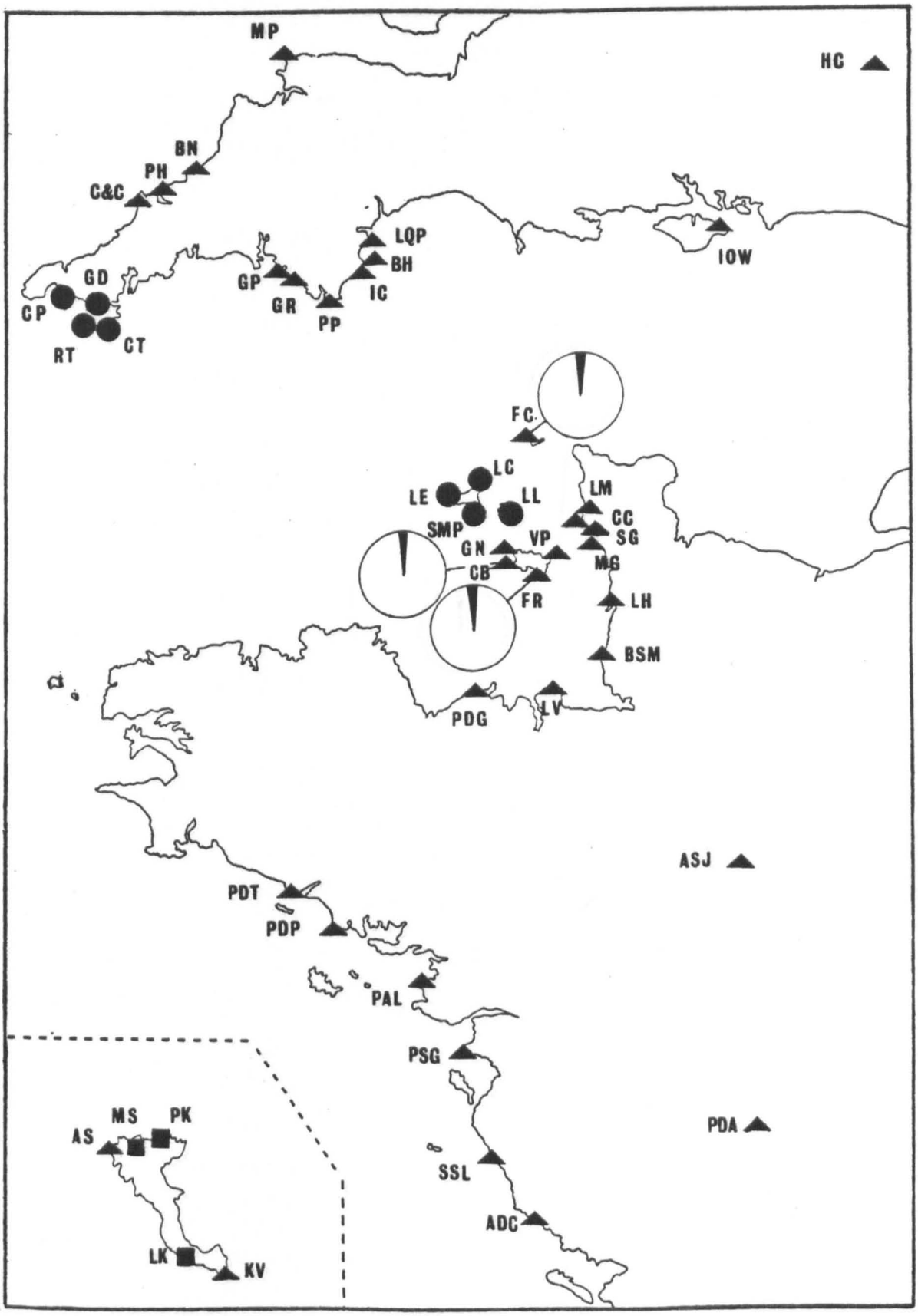
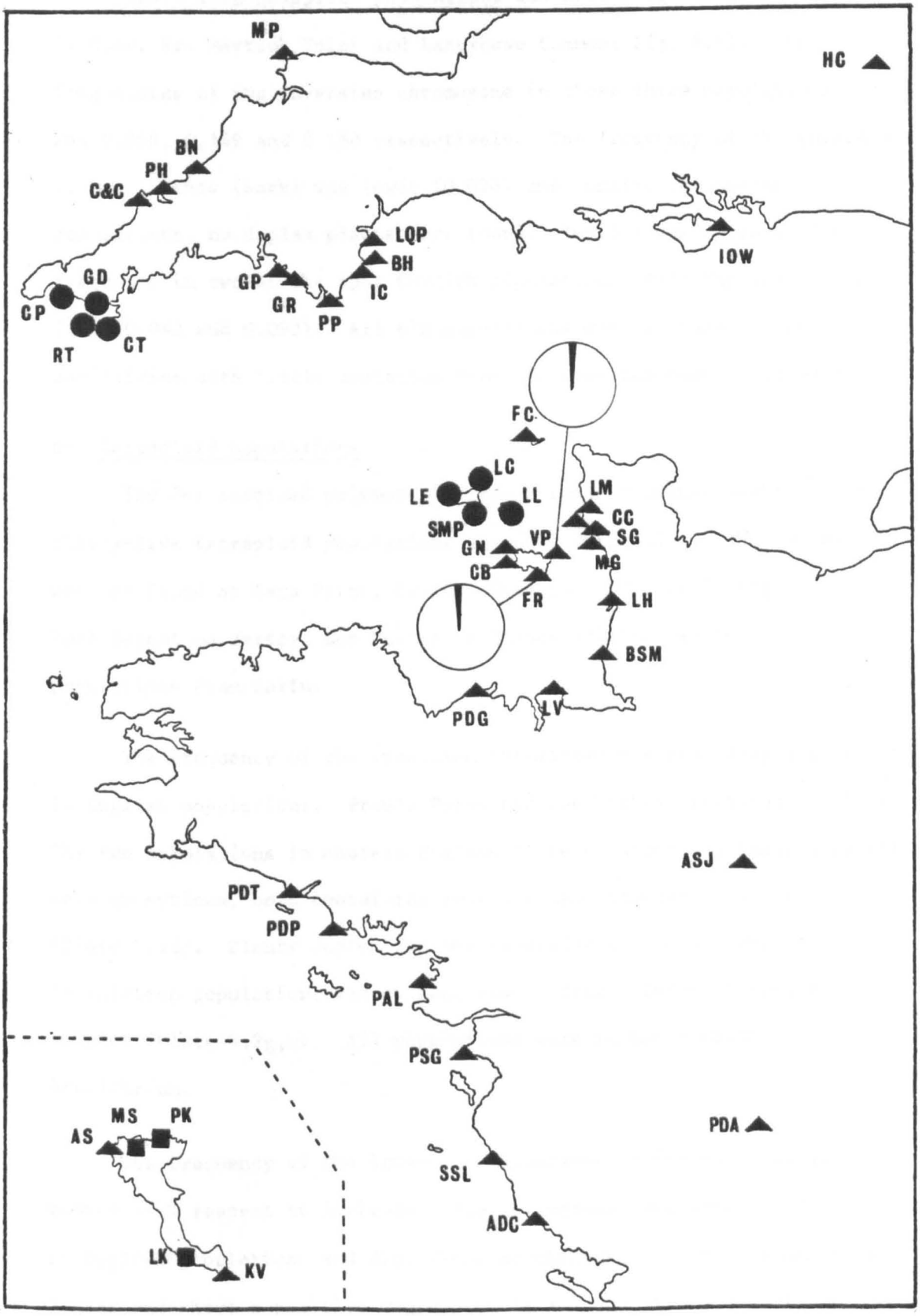


Figure 6.8 Distribution and frequency of Inv 1-4



a) Hexaploid populations

Inv 3-1 in hexaploid populations was most frequent on Guernsey (L'Eree, St. Martins Point and Lancresse Common; Fig. 6.9). The frequencies of the inversion chromosome in these three populations was 0.205, 0.169 and 0.188 respectively. The frequency of the inversion at Les 1'Aches (Sark) was lower (0.026) and, unlike the Guernsey populations, no duplex plants were found. Inv 3-1 was found at low frequency in two of the four Cornish populations, Rill Top and Caerleon Cove (0.043 and 0.092). All six populations were in Hardy-Weinberg equilibrium with little deviation from the expected morph frequencies.

b) Tetraploid populations

Inv 3-1 attained polymorphic proportions in twenty-eight of the thirty-five tetraploid populations examined (Fig. 6.10). The inversion was not found at Gara Point, Gunrow's Down and Ivy Cove in England, Fort Regent on Jersey, Les Mièles in France and the two tetraploid populations from Corfu.

The frequency of the inversion chromosome was generally higher in English populations. Prawle Point had the highest frequency of 0.18. The two populations in eastern England (Isle of Wight and Hampton Court) were exceptions, both containing only a single simplex individual (Plate 6.2d). Plants duplex for the inversion chromosome were found in thirteen populations and at Long Quarry Point (Devon) a single triplex (Plate 6.2g,h). All populations were in Hardy-Weinberg equilibrium.

The frequency of the inversion chromosome varies in a clinal manner with respect to latitude. The chromosome frequency is greatest in English populations and diminishes southwards. The relationship is linear and shows a positive regression ($t = 3.196$, $P < 0.01$; Fig. 6.11a).

Figure 6.9 Distribution and frequency of Inv 3-1 in autoallohexaploids

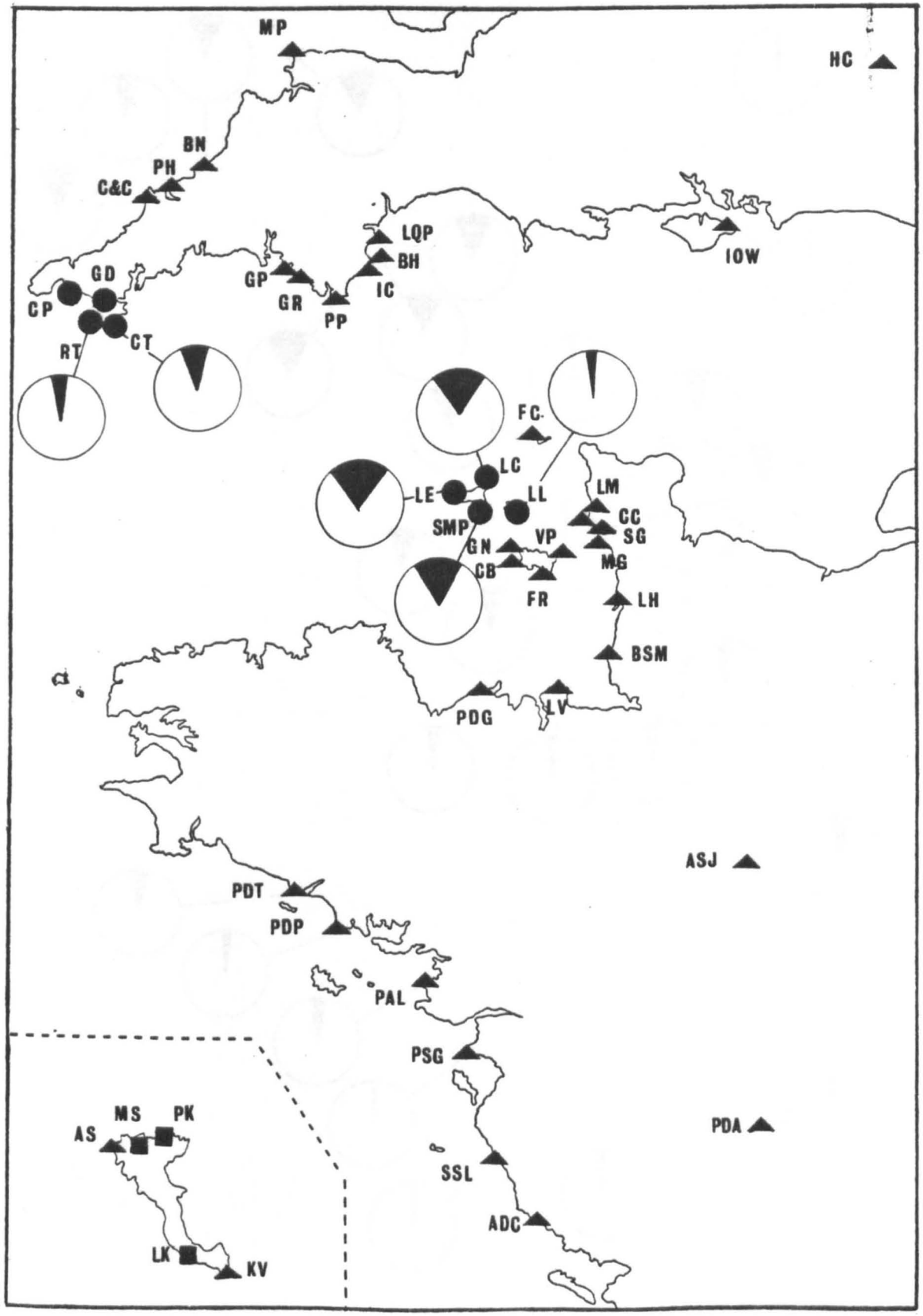


Figure 6.10 Distribution and frequency of Inv 3-1 in autotetraploids

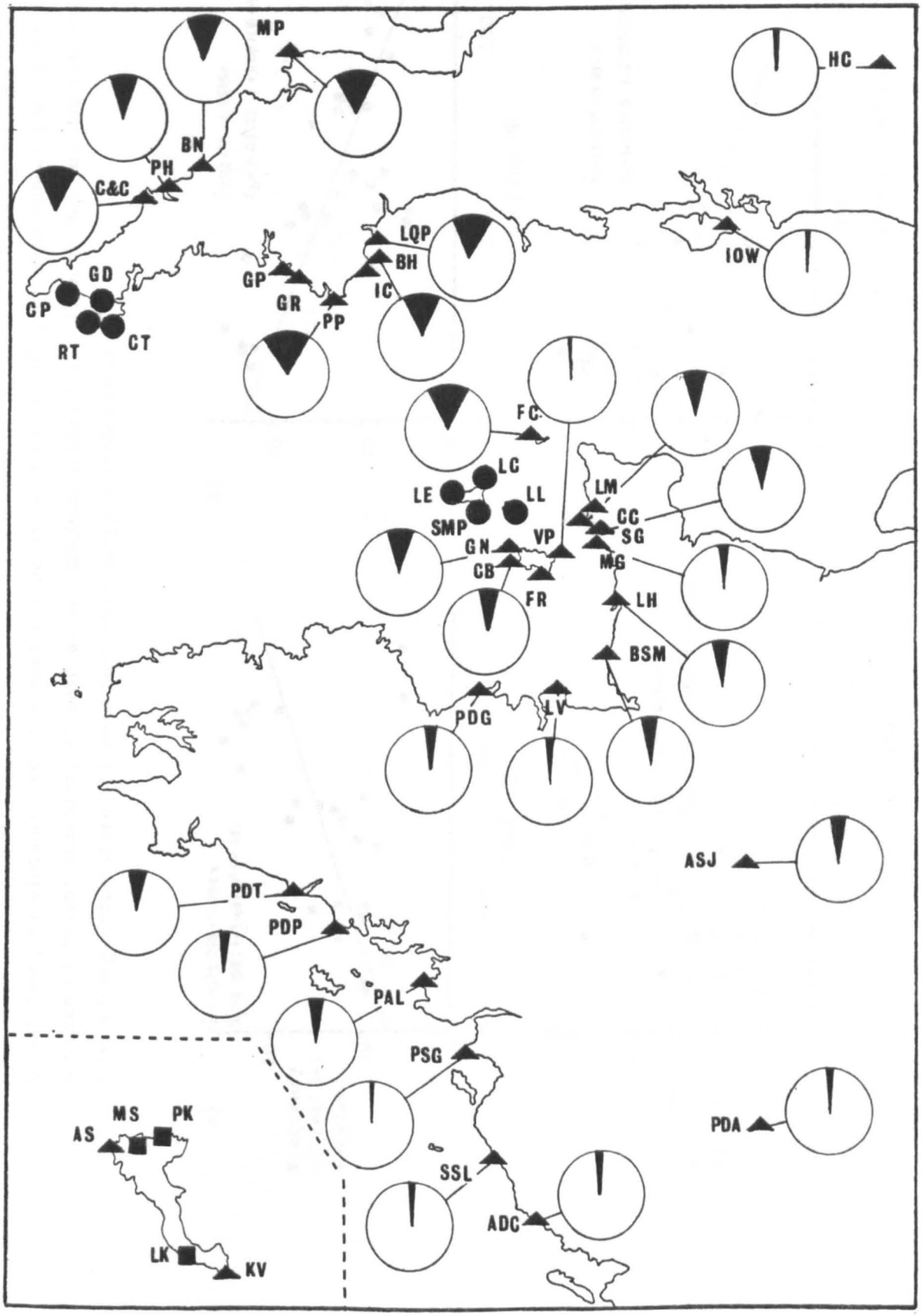


Figure 6.11 Regression of the frequency of Inv 3-1 chromosomes in autotetraploid populations of S. autumnalis against a) latitude, b) distance from the most northerly population (MP), c) mean annual precipitation, d) mean duration of bright sunshine, e) mean annual maximum temperature, f) mean May maximum temperature, g) mean July maximum temperature, h) mean September maximum temperature.

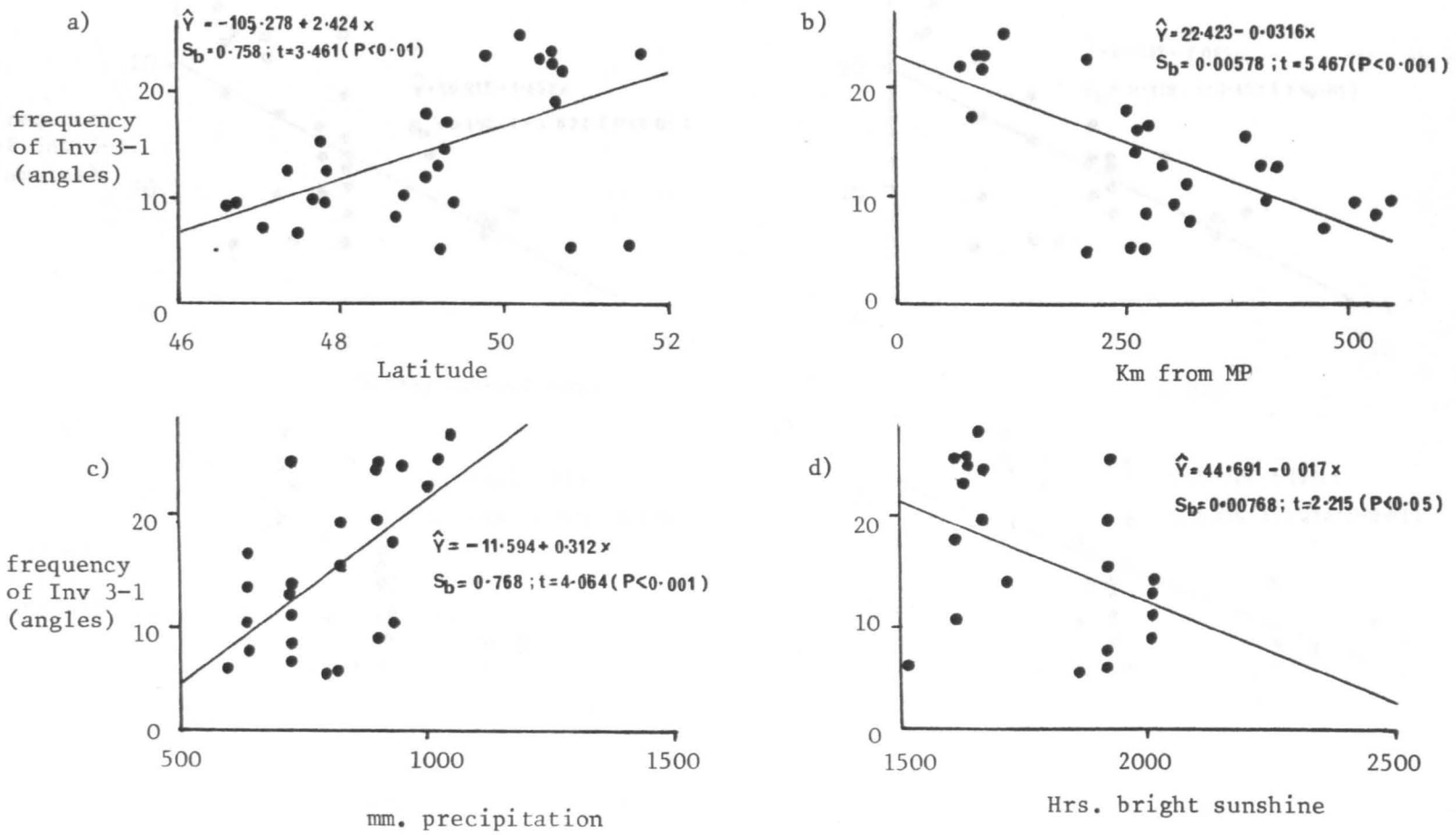
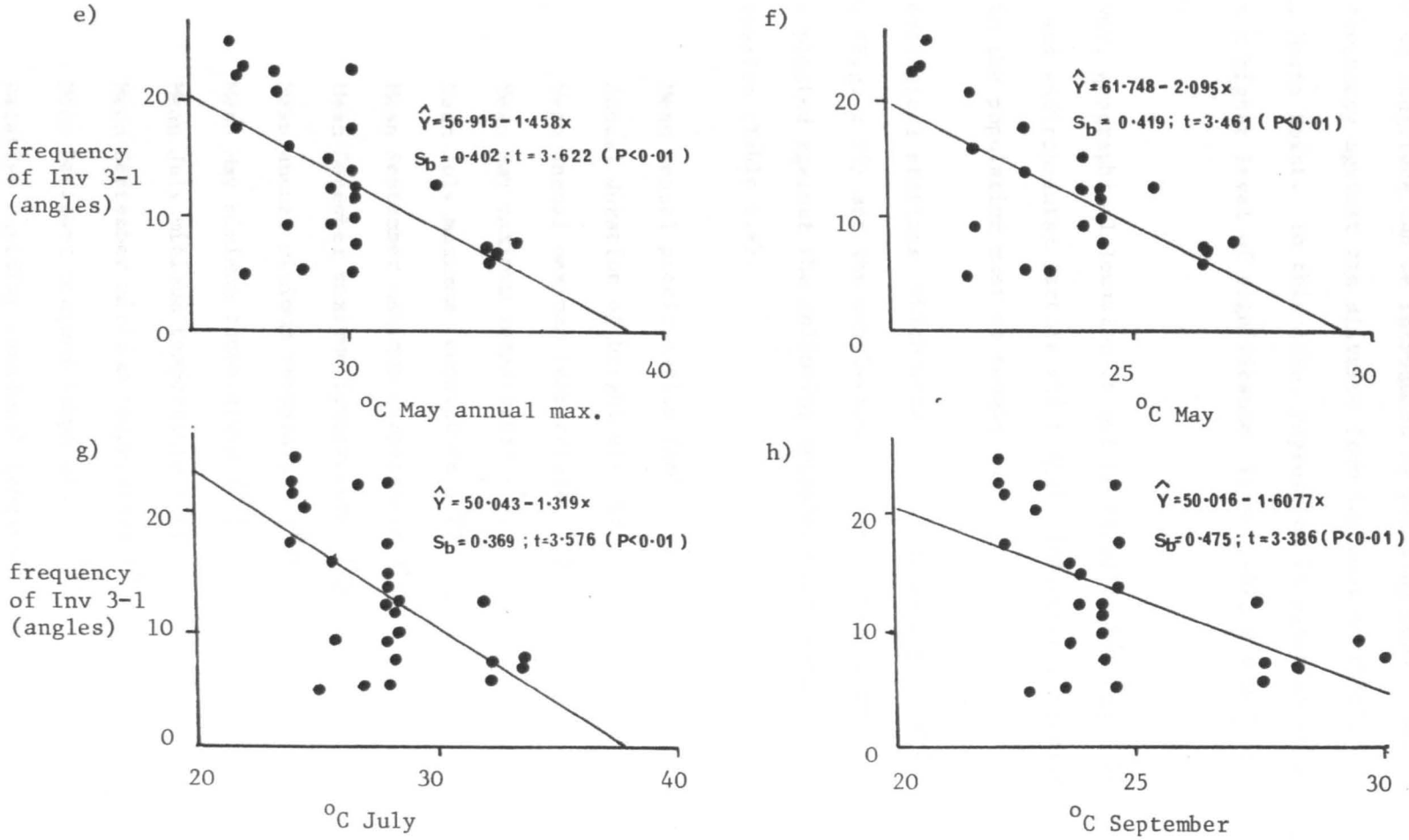


Figure 6.11 continued



A component of longitude can be introduced by plotting population inversion frequency against the distance from the most northerly population, Morte Point. In this case, regression is again positive but reaches a higher level of significance ($t = 5.467$, $P < 0.001$; Fig 6.11b).

However, geographical location is not necessarily biologically meaningful and environmental factors which might influence inversion frequency in the population must be sought.

Meteorological stations which were closest to each population were chosen (Fig. 6.12) and the population inversion chromosome frequencies plotted against the following meteorological data from each station (Table 6.4).

Mean annual precipitation (mm)
Annual duration of bright sun (hrs)
Mean annual maximum temperature ($^{o}C$)
Mean May maximum temperature ($^{o}C$)
Mean July maximum temperature ($^{o}C$)
Mean September maximum temperature ($^{o}C$)
Mean December maximum temperature ($^{o}C$)
Mean annual minimum temperature ($^{o}C$)
Mean May minimum temperature ($^{o}C$)
Mean July minimum temperature ($^{o}C$)
Mean September minimum temperature ($^{o}C$)
Mean December minimum temperature ($^{o}C$)
Relative humidity (maximum) (Angles)
Relative humidity (minimum) (Angles)

(Fig. 6.11).

Figure 6.12 Meteorological stations in southern England and north-western France (open circles) and populations associated with them for analysis of clinal variation in Inv 3-1 (see Fig. 6.11).

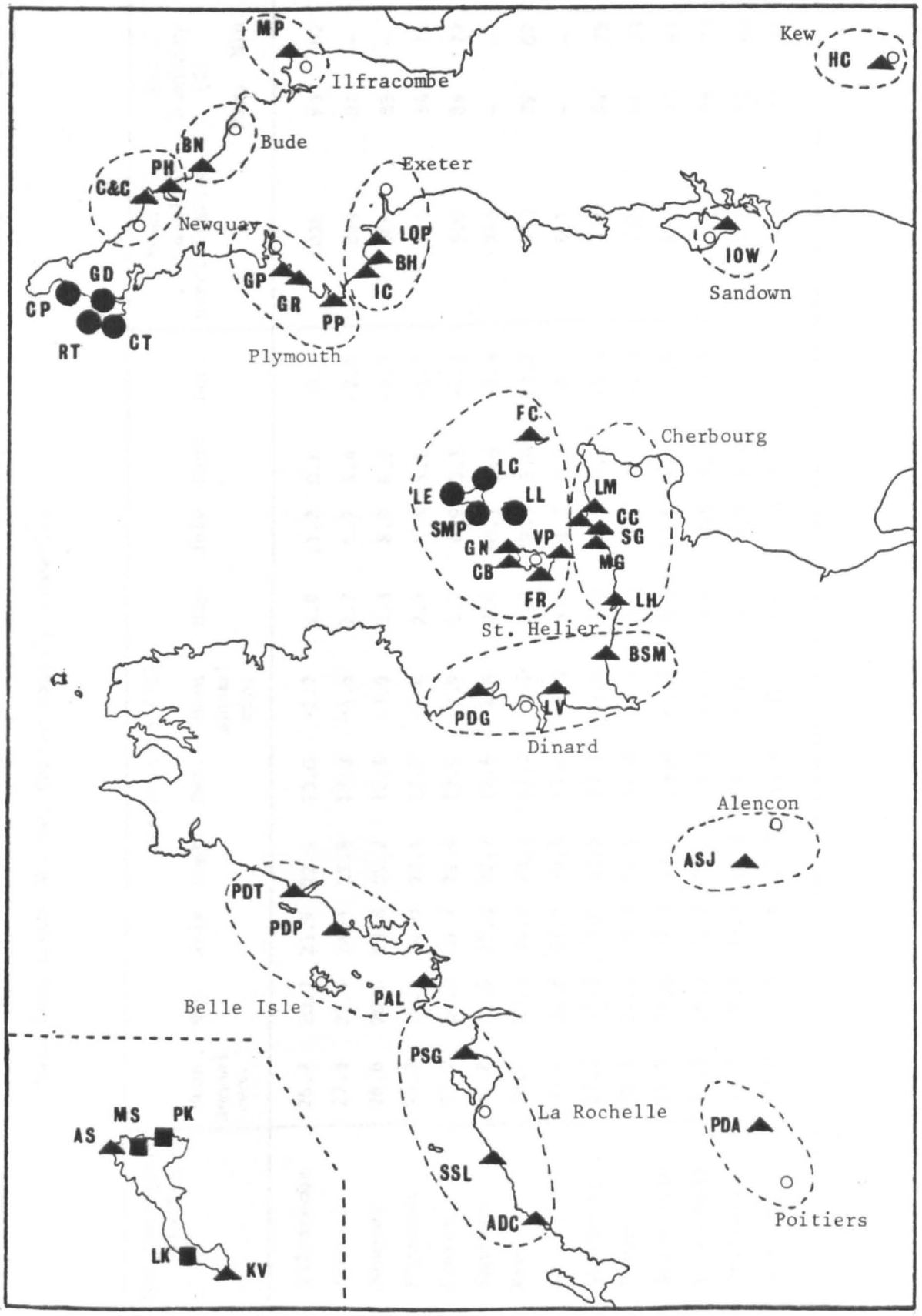


Table 6.4 Meteorological data from stations in southern England, the Channel Islands and north-western France.

Data from London Weather Centre monthly summaries

| Meteorological Station | Temperature (°C) | | | | | | | | | | Mean annual precipitation | Rel. humidity (%) | | Duration of bright sunshine (hrs) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean annual max. | May | July | Sept. | Dec. | Mean annual min. | May | July | Sept. | Dec. | | Max. | Min. | |
| Ilfracombe | 26.7 | 20.7 | 23.9 | 22.1 | 13.0 | -2.7 | 4.8 | 11.2 | 8.8 | 0.1 | 1026 | 83 | 82 | 1618 |
| Bude | 27.8 | 21.7 | 24.4 | 22.8 | 13.3 | -5.6 | 1.7 | 6.7 | 4.4 | -2.8 | 869 | 81 | - | 1625 |
| Newquay | 26.6 | 20.0 | 23.8 | 22.2 | 12.8 | -3.9 | 3.3 | 8.9 | 6.1 | -1.1 | 866 | 85 | - | 1664 |
| Plymouth | 26.3 | 20.8 | 24.0 | 22.1 | 12.7 | -4.6 | 2.9 | 8.4 | 5.8 | -1.7 | 950 | 84 | 76 | 1657 |
| Exeter | 27.7 | 20.5 | 26.7 | 22.9 | 13.6 | -7.9 | 1.2 | 6.9 | 3.1 | -4.1 | 800 | 84 | 72 | 1636 |
| Sandown | 26.8 | 21.6 | 25.0 | 22.7 | 12.6 | -4.6 | 3.4 | 10.1 | 6.6 | -1.8 | 769 | - | - | 1858 |
| Kew | 28.6 | 23.3 | 26.9 | 23.3 | 12.3 | -5.7 | 2.7 | 9.3 | 5.4 | -3.2 | 593 | 79 | 67 | 1514 |
| St. Helier | 30.2 | 22.8 | 27.9 | 24.5 | 13.0 | -2.4 | 5.9 | 11.7 | 9.6 | 0.2 | 801 | - | - | 1917 |
| Cherbourg | 28.2 | 21.8 | 25.6 | 23.5 | 13.3 | -3.4 | 5.4 | 10.9 | 9.2 | -0.4 | 931 | 84 | 75 | 1608 |
| Dinard | 30.3 | 24.4 | 28.2 | 24.2 | 14.0 | -5.4 | 3.3 | 8.5 | 7.2 | -1.4 | 726 | 91 | 73 | 2007 |
| Belle Isle | 29.5 | 24.0 | 27.9 | 23.7 | 13.8 | -3.8 | 5.7 | 10.5 | 9.5 | 0.9 | 637 | 93 | 85 | - |
| La Rochelle | 34.4 | 26.5 | 32.1 | 27.5 | 14.3 | -6.7 | 4.0 | 10.8 | 8.0 | -3.5 | 727 | 88 | 72 | - |
| Poitiers | 34.7 | 26.6 | 33.4 | 28.2 | 14.2 | -10.1 | 1.8 | 8.0 | 5.0 | -5.4 | 645 | 92 | 68 | 1916 |
| Alencon | 32.8 | 25.5 | 31.8 | 27.4 | 12.9 | -11.7 | 0.5 | 5.3 | 2.9 | -0.2 | 724 | 93 | 71 | 1712 |

Regression analysis shows that of the 14 meteorological variables plotted against the inversion chromosome frequency, six are significant (Table 6.5). The highest level of significance was given by mean annual precipitation ($t = 4.064$, $P < 0.001$). Mean annual maximum temperature ($t = 3.622$) and the mean monthly temperatures for May ($t = 3.461$), July ($t = 3.576$) and September ($t = 3.386$) all gave significant negative regressions at the 1% level. The annual duration of bright sun was significant at the 5% level ($t = 2.215$). Mean annual precipitation, mean maximum temperature and the duration of bright sun are clearly related variables and indicate that the individuals carrying Inv 3-1 chromosomes are found preferentially in areas of cooler and wetter summer climate (the period of growth). More precise micrometeorological data is required before one particular facet of climate can be identified.

Pollen stainability estimates (Table 6.6) show that both duplex (83%) and simplex (80%) plants have significantly higher values than nulliplex individuals (71%) ($F = 3.940$, $P < 0.05$; Table 6.7).

Inv 3-2

Inv 3-2, a pericentric B3 inversion, shifted the centromere to a more median position giving an arm ratio of 1.4 (Fig. 5.2). The inversion did not include the nucleolar-organiser. Inv 3-2 was limited to a single French population (Pont de l'Argenton; Fig. 6.1) with three simplex plants in 36.

Inv 3-3

Inv 3-3, a paracentric B3 inversion, shifted the nucleolar organiser to a more distal location (Fig. 5.2). This inversion was found in two simplex individuals of the Le Verger, France population (chromosome frequency 0.017; Fig. 5.2, Plate 6.2j).

Table 6.5 Regression analysis of Inv 3-1 frequency in tetraploid populations of *S. autumnalis* against fourteen meteorological variables and two geographical parameters

| Variable/Parameter | b | $S_b$ | t | P |
|---|---|---|---|---|
| Latitude (degrees) | 2.424 | 0.758 | 3.196 | <0.01 |
| Distance from Morte Point (km) | 0.0316 | 0.00578 | 5.467 | <0.001 |
| Mean annual precipitation (mm) | 0.312 | 0.00768 | 4.064 | <0.001 |
| Annual duration of bright sun (hrs) | -0.017 | 0.00768 | 2.215 | <0.05 |
| Mean annual maximum temp. ($^{o}$C) | -1.458 | 0.402 | 3.622 | <0.01 |
| Mean May maximum temp | -2.095 | 0.419 | 3.461 | <0.01 |
| Mean July maximum temp. | -1.319 | 0.369 | 3.576 | <0.01 |
| Mean Sept. maximum temp. | -1.608 | 0.475 | 3.386 | <0.01 |
| Mean Dec. maximum temp. | 2.721 | 1.523 | 1.787 | >0.05 |
| Mean annual minimum temp. ($^{o}$C) | 0.557 | 0.522 | 1.068 | >0.2 |
| Mean May minimum temp. | -0.344 | 0.760 | 0.453 | >0.7 |
| Mean July minimum temp. | -0.586 | 0.711 | 0.825 | >0.4 |
| Mean Sept. minimum temp. | 0.544 | 0.686 | 0.793 | >0.4 |
| Mean Dec. minimum temp. | 0.543 | 0.686 | 0.793 | >0.4 |
| Relative humidity (max) (Angles) | -0.691 | 0.310 | 1.448 | >0.1 |
| Relative humidity (min) | 0.719 | 0.368 | 1.93 | >0.05 |

Table 6.6 Pollen stainability in tetraploid plants of S. autumnalis containing different numbers of Inv 3-1 chromosomes

| Inv 3-1 karyotype | Pollen stainability (%) mean | s.d. | No. of plants |
|---|---|---|---|
| Inv 3-1 nulliplex | 71.33 | 14.65 | 47 |
| Inv 3-1 simplex | 80.33 | 8.11 | 19 |
| Inv 3-1 duplex | 82.83 | 4.93 | 10 |
| Inv 3-1 triplex | 76.14 | 5.51 | 2 |

Table 6.7 Analysis of variance of pollen stainability in tetraploid plants of S. autumnalis containing different numbers of Inv 3-1 chromosomes (after angular transformation)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between karyotypes | 3 | 1787.802 | 595.934 | 3.910 | <0.05 |
| Between plants | 74 | 11278.065 | 152.406 | | |
| Total | 77 | 13065.867 | | | |

Inv 3-4

The pericentric inversion Inv 3-4 shifted the centromere to a slightly more acrocentric position without affecting the long arm NOR - telomere distance. This inversion was confined to the Gara Point population in which two simplex plants were found (inversion chromosome frequency 0.017; Figs.5.2 and 6.1).

Inv 3-5

In Inv 3-5 the centromere was shifted to a nearly median position giving an arm ratio of 1.2 (Fig. 5.2). This inversion was confined to the Corbiere population in which two plants simplex for the inversion chromosome were found (frequency 0.016; Fig. 6.1; Plate 6.2k).

Inv 3-6

The paracentric inversion Inv 3-6 was recognisable since the nucleolar-organiser was moved to a slightly more distal location (Fig. 5.2). The inversion was limited to two populations on Corfu, Aghios Stephanos and Kavos, where the frequencies of the inversion chromosome were 0.076 and 0.052 respectively (Fig. 6.13). Both simplex and duplex plants being present in both populations (Plate 6.2 l,m).

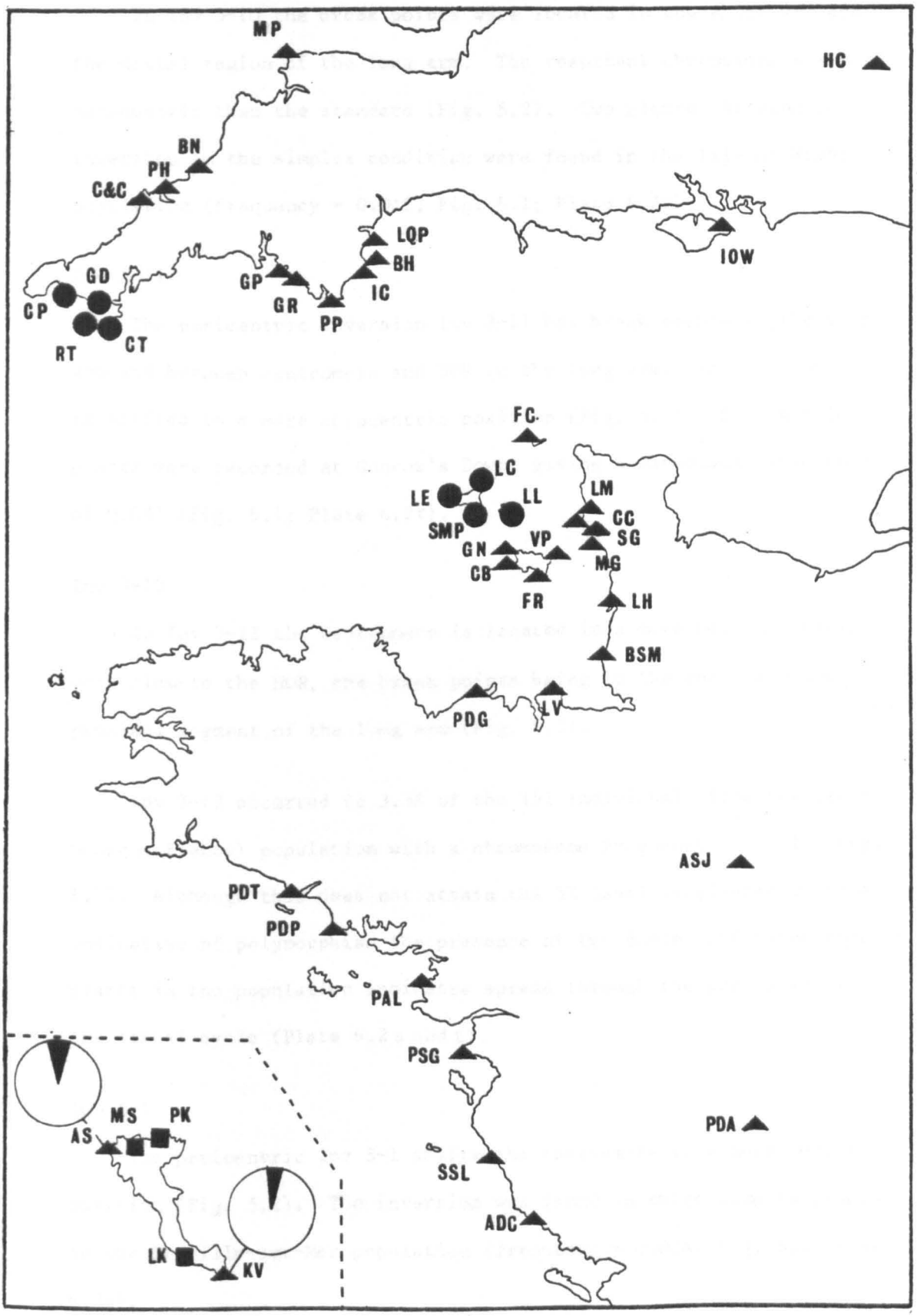
Inv 3-7

Inv 3-7, a pericentric inversion for B3, caused a shift in the centromere to a more median position but did not affect the NOR (Fig. 5.2). Two simplex plants were found in Aghios Stephanos, Corfu (frequency 0.022; Fig. 6.1; Plate 6.2n).

Inv 3-8

This paracentric inversion (Inv 3-8) caused a slight distal shift of the NOR (Fig. 5.2). The inversion chromosome was found in two simplex and one duplex plants at Aghios Stephanos, a chromosome frequency of 0.039 (Fig. 6.1; Plate 6.2).

Figure 6.13 Distribution and frequency of Inv 3-6



Inv 3-10

In Inv 3-10 the break points were located in the short arm and the distal region of the long arm. The resultant chromosome was more metacentric than the standard (Fig. 5.2). Two plants carrying this inversion in the simplex condition were found in the Isle of Wight population (frequency = 0.015; Fig. 6.1; Plate 6.2q).

Inv 3-11

The pericentric inversion Inv 3-11 has break points in the short arm and between centromere and NOR in the long arm. The centromere is shifted to a more acrocentric position (Fig. 5.2). Four simplex plants were recorded at Gunrow's Down, giving a chromosome frequency of 0.031 (Fig. 6.1; Plate 6.2r).

Inv 3-12

In Inv 3-12 the centromere is located in a more median location very close to the NOR, the break points being in the short arm and proximal segment of the long arm (Fig. 5.2).

Inv 3-12 occurred in 3.3% of the 151 individuals from the Saint Georges(France) population with a chromosome frequency of 0.012 (Fig. 6.1). Although this does not attain the 5% level considered above as indicative of polymorphism the presence of two duplex and three simplex plants in the population indicates spread through the population via the sexual cycle (Plate 6.2 s and t).

Inv 5-1

The pericentric Inv 5-1 shifts the centromere to a more median position (Fig. 5.2). The inversion was found in three simplex plants in the Breville-sur-Mer population (frequency = 0.024, Fig. 6.1; Plate 6.2u).

Inv 6-1

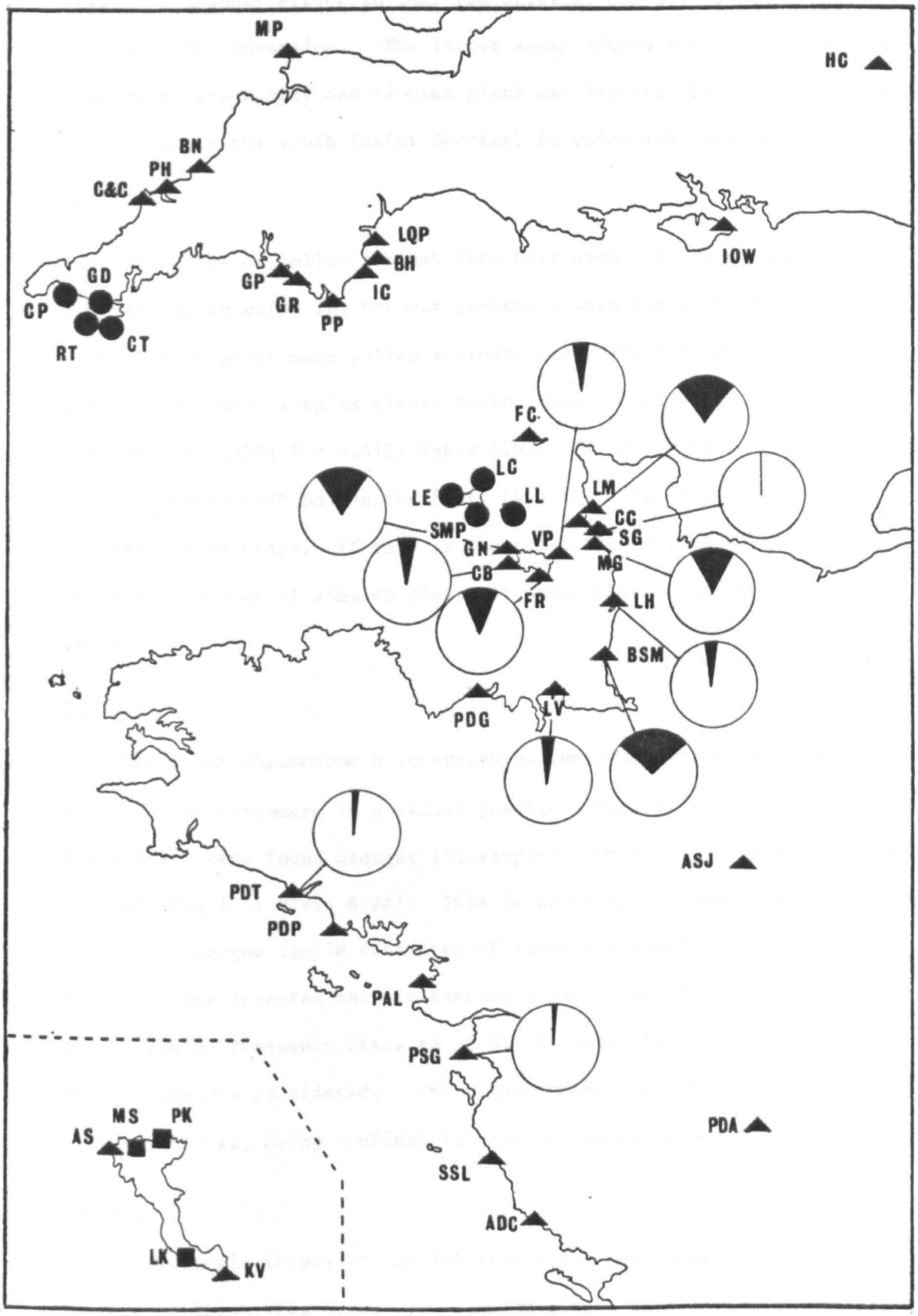
In Inv 6-1 the centromere is much more acrocentrically positioned (Fig. 5.2). This polymorphism is extremely widespread occurring in twelve tetraploid populations through north western France and the Channel Islands (Fig. 6.14). The polymorphism is centred on the Cotentin Peninsula (Normandy) and Jersey but reaches the more southerly populations of Pointe de Talud and Pointe de St. Gildas. The polymorphism is thus distributed over 220 km.

In six populations, duplex and simplex individuals were found (Plate 6.2v and w ). The Inv 6-1 chromosome was most frequent in the BSM population (0.258). Interestingly, nulliplex plants were the minority class in this population. The fit to the Hardy-Weinberg equilibrium is close although one or two triplex plants would be expected with this size population. The discrepancy is likely to result from sampling error although triplex plants might be at a selective disadvantage. Where the frequency of the inversion is high (BSM, LM, GN) there seems to be an indication of heterozygote advantage with an excess of simplex individuals. In none of 12 populations, however, is there a great deviation from the predicted H-W value.

Though the polymorphism is widespread there is no obvious geographical trend in inversion chromosome frequency, and in some cases, neighbouring populations have widely disparate frequencies (Fig. 6.14). The selective force(s) responsible for the maintenance of this polymorphism, therefore, are not likely to be climatic but perhaps are associated with the immediate habitat of the population.

In two populations from the main geographical area of the inversion - Fort Corbelets (Alderney) and Cap Carteret (Normandy) - the inversion

Figure 6.14 Distribution and frequency of Inv 6-1



was not detected. This may simply result from sampling error or may reflect a founder effect in that the original colonising plants did not carry the inversion. The latter seems likely for the Cap Carteret population since only one simplex plant was detected in the neighbouring population to the south (Saint Georges) in which 151 individuals were scored.

Estimates of pollen stainability have been made on plants from populations in which Inv 6-1 was present (Table 6.8). Nulliplex plants showed the highest mean pollen stainability (79%) followed by duplex plants (73%) with simplex plants having significantly the lowest stainability (65%; F = 6.419; Table 6.9). If pollen stainability can be equated with pollen fertility then the simplex plants are at a clear disadvantage. If this is the case, then strong selection must operate in favour of simplex plants at some other stage of the life cycle.

Inv 6-2

The other chromosome 6 inversion polymorphism detected - Inv 6-2 - shifted the centromere to a median position (Fig. 5.2). Three simplex individuals were found amongst 151 sampled from Saint Georges (frequency = 0.005; Fig 6.1; Plate 6.2x). This is probably an underestimate since the Saint Georges sample consisted of seven sub-populations and the inversion was detected only in samples 5 and 6 containing 60 plants. The inversion frequency rises to 0.013 if only these two sub-populations are considered. The polymorphism then is extremely limited in area, being confined to part of the Saint Georges population.

Inv 7-1

Pericentric inversion Inv 7-1 shifts the centromere to an acrocentric position (Fig 5.2). Two simplex plants were found in the Pointe

Table 6.8 Pollen stainability in tetraploid plants of S. autumnalis containing different numbers of Inv 6-1 chromosomes

| Inv 6-1 karyotype | Pollen stainability (%) | | No. of plants |
|---|---|---|---|
| | Mean | s.d. | |
| Inv 6-1 nulliplex | 79.22 | 10.73 | 17 |
| Inv 6-1 simplex | 65.14 | 12.82 | 17 |
| Inv 6-1 duplex | 72.94 | 7.96 | 5 |

Table 6.9 Analysis of variance of pollen stainability in tetraploid plants of S. autumnalis containing different numbers of Inv 6-1 chromosomes (after angular transformation)

| Source | D.F. | S.S | M.S | F | P |
|---|---|---|---|---|---|
| Between karyotypes | 2 | 1681.224 | 840.612 | 6.419 | <0.01 |
| Between plants | 36 | 4714.603 | 130.961 | | |
| Total | 38 | 6395.827 | | | |

de Talud population giving a frequency of 0.017 (Fig. 6.1; Plate 6.2y).

Inv 7-2

Three individuals were detected in the Fort Corbelets population which were simplex for a B7 chromosome with a pericentric inversion (Inv 7-2, Fig. 5.2, Plate 6.2z). The inversion shifted the centromere to a more acrocentric location.

Duplication Polymorphisms

Dup 1-1

Dup 1-1 increases the length of the short arm of chromosome 1 by 1.2 μm (51%; Fig. 5.3). This duplication reaches polymorphic proportions in four adjacent populations in South Devon and simplex and duplex individuals are found here (Fig. 6.15, Plate 6.3 a and b ). The frequency of this chromosome reaches a maximum of 0.156 in the Berry Head population and declines in frequency in populations to the north and the south, perhaps indicating its point of origin. All four populations are in Hardy-Weinberg equilibrium. This variant chromosome, then, reaches a high frequency over 35 km.

Dup 1-2

A long arm duplication of chromosome (Dup 1-2) increases the length by 1.3 μm (Fig. 5.3). The duplication reaches polymorphic proportions in four populations in France (Pointe du Percho, Le Verger, Pointe de Talud and Sion-sur-L'Ocean; Fig. 6.16). In addition single duplication chromosomes were found in three further populations (Pointe de St. Gildas, Anse de Cayola and Breville-sur-mer, Plate 6.3c). Duplex individuals were uncommon (Plate 6.3d).

This polymorphism is restricted to coastal populations in France, the populations with the highest frequency of Dup 1-2 being most abundant

Figure 6.15 Distribution and frequency of Dup 1-1

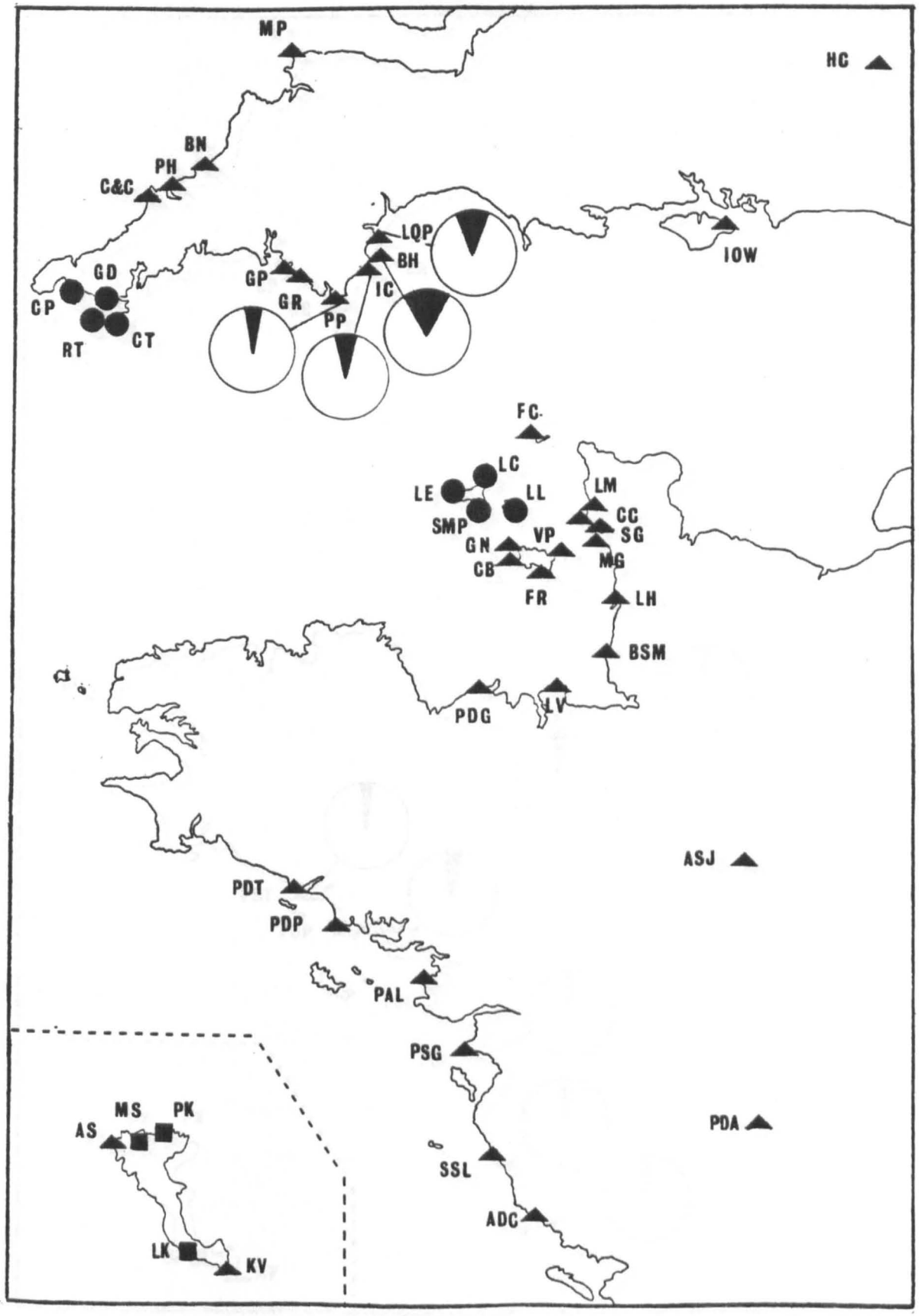
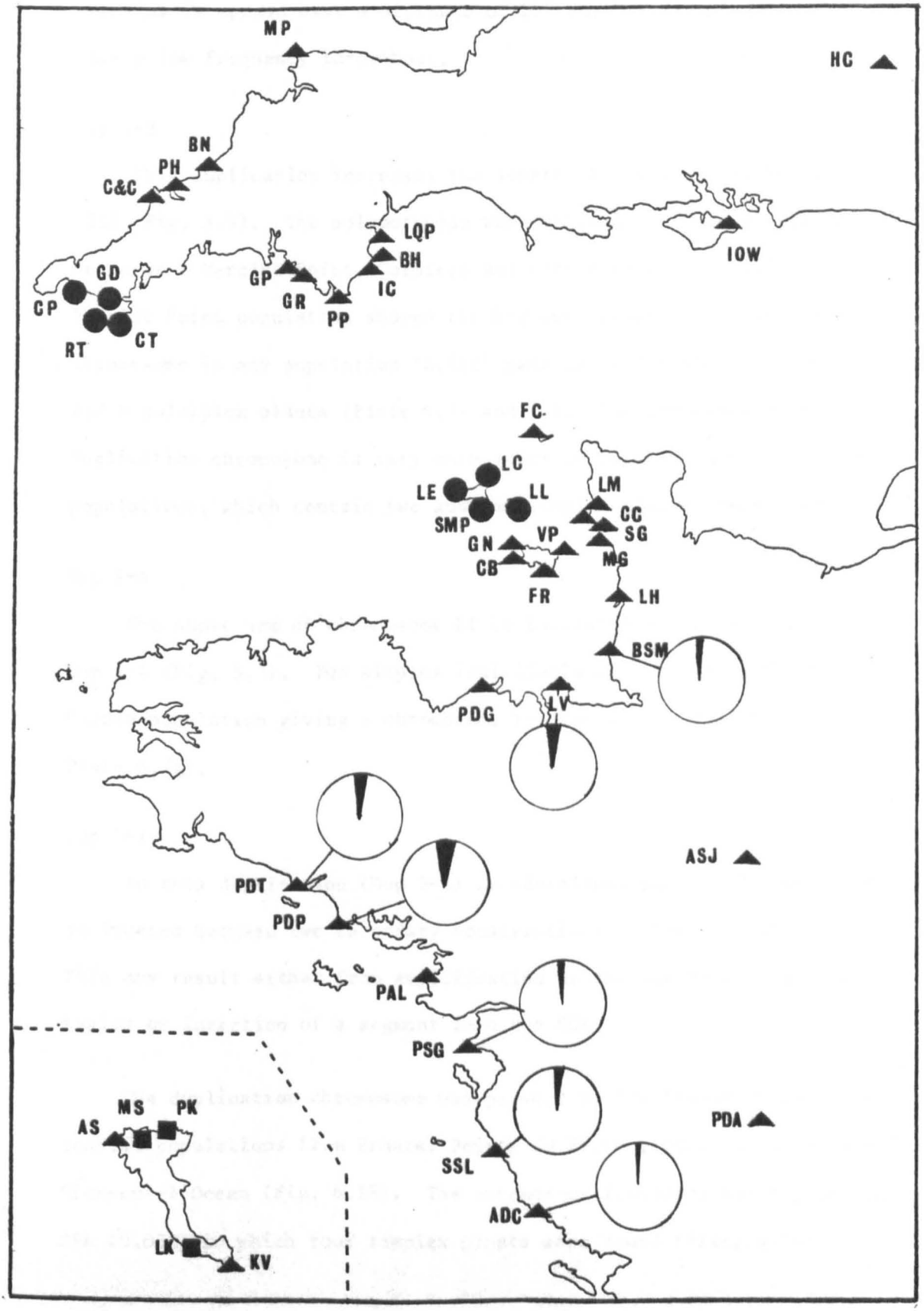


Figure 6.16 Distribution and frequency of Dup 1-2



in southern Brittany and declining to the north and south. The polymorphism is spread over a distance of 220 km, but is maintained at a fairly low frequency throughout.

Dup 1-3

This duplication increases the length of the long arm by 1.5 μm (25%; Fig. 5.3). The polymorphism was confined to three populations on Jersey: Verclut Point, Corbiere and Fort Regent (Fig. 6.17). The Verclut Point population showed the highest frequency of any duplication chromosome in any population (0.242) made up of 7 duplex, 16 simplex and 8 nulliplex plants (Plate 6.3e and f ). The frequency of the duplication chromosome is very much lower in Corbiere and Fort Regent populations, which contain two and one simplex plants respectively.

Dup 1-4

The short arm of chromosome B1 is increased by 0.6 μm (25%) in Dup 1-4 (Fig. 5.3). Two simplex individuals were found in the Anse de Cayola population giving a chromosome frequency of 0.018 (Fig. 6.2; Plate 6.3g).

Dup 3-1

In this duplication (Dup 3-1) an additional segment 0.7 μm in length is located between two secondary constrictions in the long arm (Fig. 5.3). This may result either from amplification of the nucleolar-organiser region or insertion of a segment into the NOR.

The duplication chromosome was present at low frequency in three coastal populations from France: Pointe du Percho, Port au Loup and Sion-sur-l'Ocean (Fig. 6.18). The chromosome frequency was highest in SSL (0.032) in which four simplex plants were found (Plate 6.3n).

Figure 6.17 Distribution and frequency of Dup 1-3

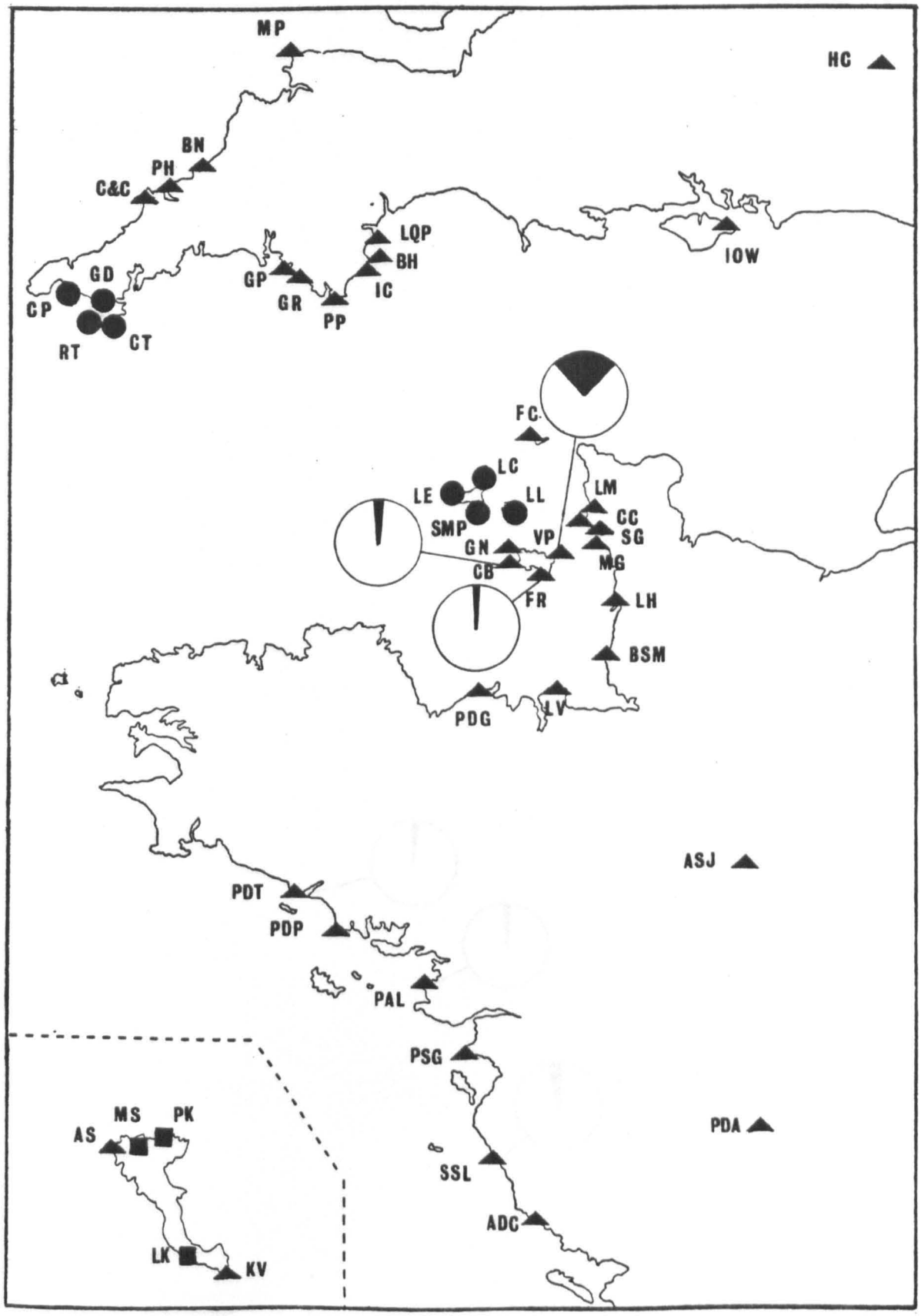
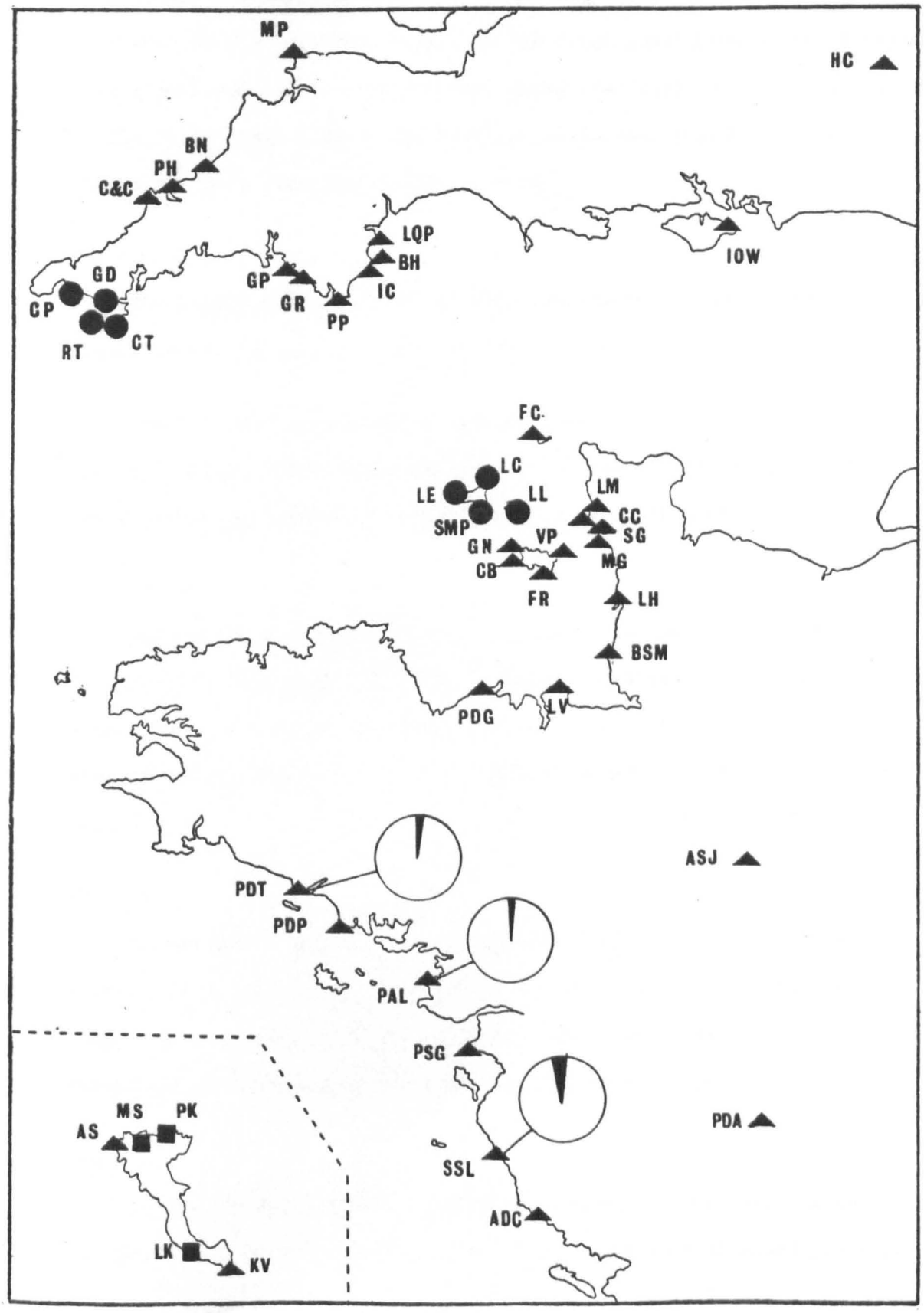


Figure 6.18 Distribution and frequency of Dup 3-1



Dup 3-2

In Dup 3-2 the length of the distal segment of the long arm was increased by 0.3 μm (Fig. 5.3). In the large population of St. Georges four plants simplex for Dup 3-2 were found (two each in samples 3 and 6; Fig. 6.2; Plate 6.3o). The duplication is thus present in the population at a very low frequency (0.007).

Dup 3-4

The long arm region of B3 between centromere and nucleolar-organiser was increased by 0.4 μm (10%) in Dup 3-4 (Fig. 5.3).

Dup 3-4 reached polymorphic proportions in the Verclut Point (Jersey) population in which five of the 31 plants were simplex for the duplication (frequency = 0.040; Fig. 6.2; Plate 6.3p).

Dup 3-5

In Dup 3-5 the length of the long arm of B3 distal to the NOR was increased by 1 μm (25%; Fig. 5.3). Dup 3-5 attained polymorphic proportions in a single population, Kavos (Corfu, Fig. 6.2). Two simplex and one duplex plant were found (frequency = 0.044; Plate 6.3q and r).

Dup 3-6

In the Kavos population, a chromosome duplication (Dup 3-6) was present which increased the length of the short arm by 0.6 μm. Two simplex plants were found in a sample of 28 (Plate 6.3s). The frequency of duplication 3-6 in the population was 0.022.

Dup 3-9

In Dup 3-9 the length of the distal segment of the long arm was increased by 0.9 μm (21%; Fig. 5.3). This chromosome attained polymorphic

proportions in a single population, Plage de Guen in France (Fig. 6.2). A duplex and a simplex were found (frequency = 0.029; Plate 6.3 u and v).

Dup 3-10

The Plage de Guen population was polymorphic for a B3 duplication (Dup 3-10) which increased the length of the centromere - NOR segment in the long arm by 0.7 µm (18%; Figs.5.3 and 6.2). Four simplex individuals were found in a population sample of 26 (chromosome frequency 0.039 Plate 6.3w).

Dup 4-1

In Dup 4-1 the length of the long arm was increased by 1.9 µm (36%; Fig. 5.3). This chromosome was detected in 6 populations: one from Jersey, three coastal and two inland French populations (Fig. 6.19). The frequency of the Dup 4-1 chromosome was always very low and at most only two simplex individuals were detected in a single population (Le Verger, Corbiere; Plate 6.3x).

Dup 5-1

Dup 5-1 has a long arm increased by 2.3 µm (51%; Fig. 5.3). This duplication was confined to a single population, Saint Georges in France (Fig. 6.2). Three simplex individuals were found in different samples of this large population (Plate 6.3y). The overall frequency of the inversion chromosome in the population was thus very low (0.005).

Dup 5-2

Dup 5-2 a B5 duplication which increased the length of the long arm by 2.9 µm (49%; Fig. 5.3) attained polymorphic proportions in the two tetraploid populations from Corfu (Kavos and Aghios Stephanos; Fig. 6.20). Simplex and duplex plants were found in both populations

Figure 6.19 Distribution and frequency of Dup 4-1

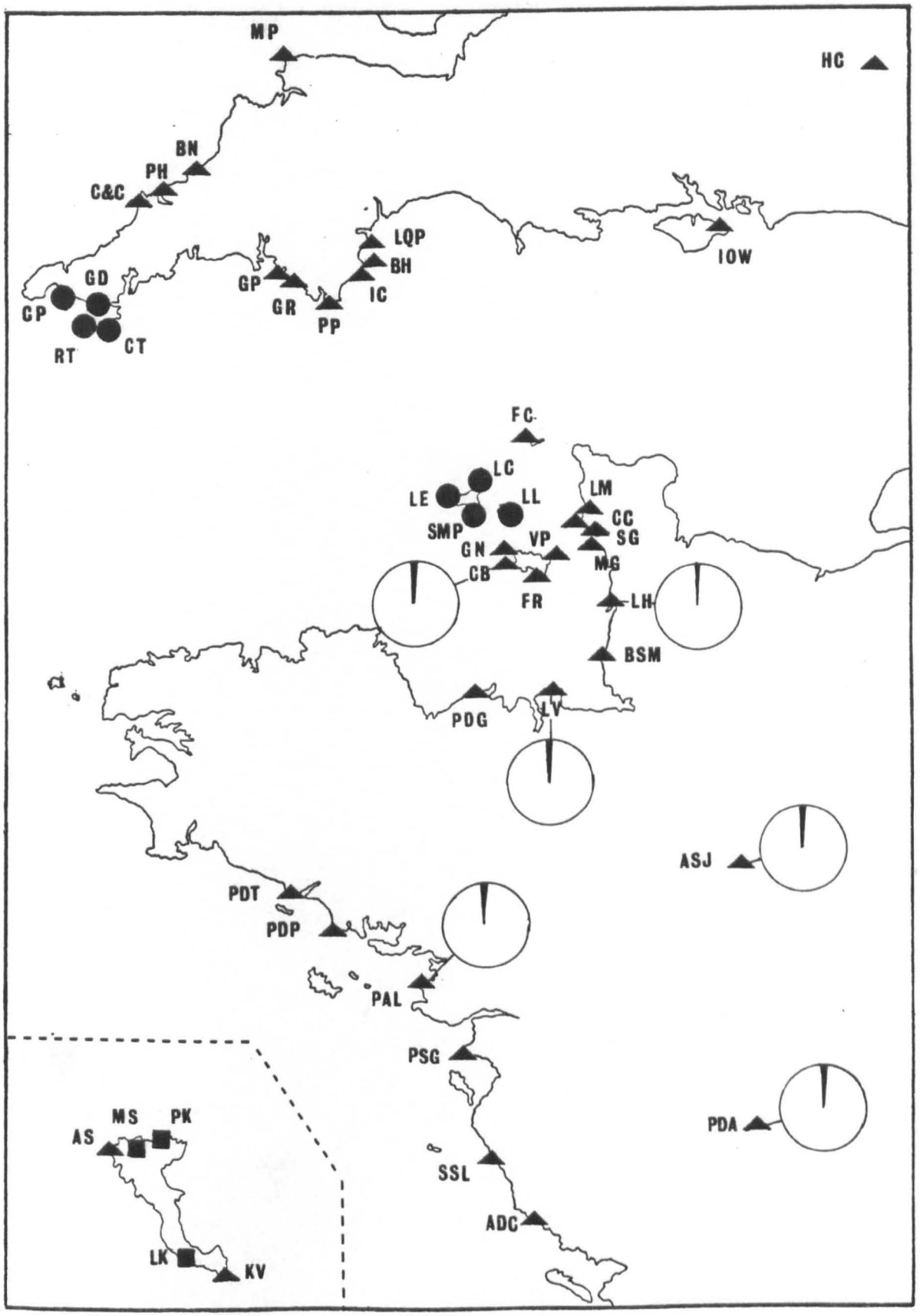
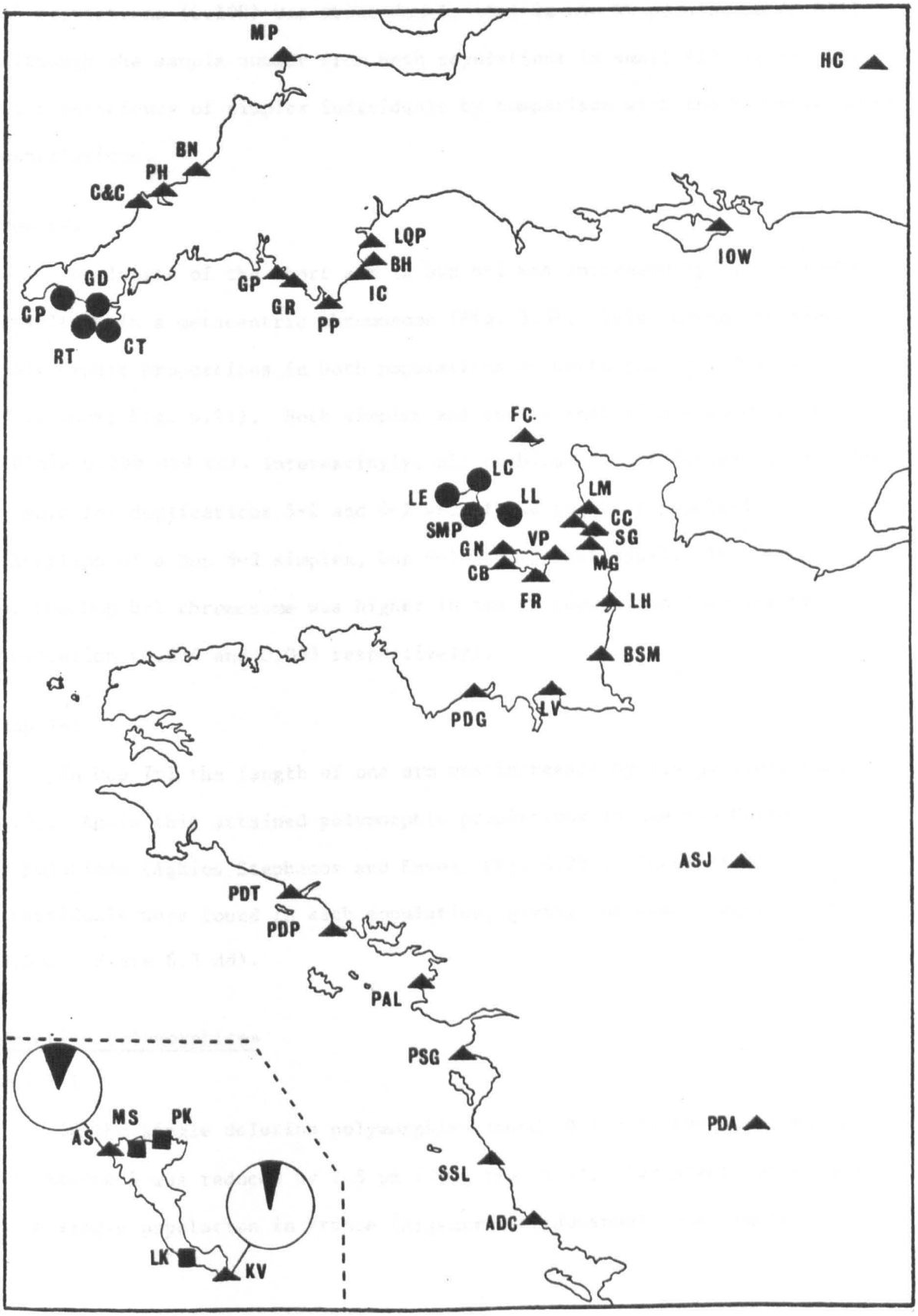


Figure 6.20 Distribution and frequency of Dup 5-2



(Plate 6.2z and aa). The frequency of the duplication chromosome in the AS populations (0.109) was about double that in the KV population (0.054). Although the sample number from both populations is small (23 plants) there is a deficiency of simplex individuals by comparison with the Hardy-Weinberg expectations.

Dup 6-1

The length of the short arm in Dup 6-1 was increased by 1.8 μm (88%) resulting in a metacentric chromosome (Fig. 5.3). This chromosome reached polymorphic proportions in both populations on Corfu (Kavos and Aghios Stephanos; Fig. 6.21). Both simplex and duplex individuals were found (Plate 6.3bb and cc). Interestingly, all combinations of duplex and simplex plants for duplications 5-2 and 6-1 were found in these populations with the exception of a Dup 5-2 simplex, Dup 6-1 duplex individual. The frequency of the Dup 6-1 chromosome was higher in the AS population than the KV population (0.054 and 0.033 respectively).

Dup 7-1

In Dup 7-1 the length of one arm was increased by 1.4 μm (56%; Fig. 5.3). Again this attained polymorphic proportions in the two Corfu populations (Aghios Stephanos and Kavos; Fig. 6.22). Three simplex individuals were found in each population, giving the same frequency of 0.033 (Plate 6.3 dd).

Deletion Polymorphisms

Del 5-1

In the single deletion polymorphism found, Del 5-1, the long arm of chromosome 5 was reduced by 2.5 μm (55%; Fig. 5.1). Two plants were found in a single population in France (Argentré-sur-Jouanne), one simplex and

Figure 6.21 Distribution and frequency of Dup 6-1

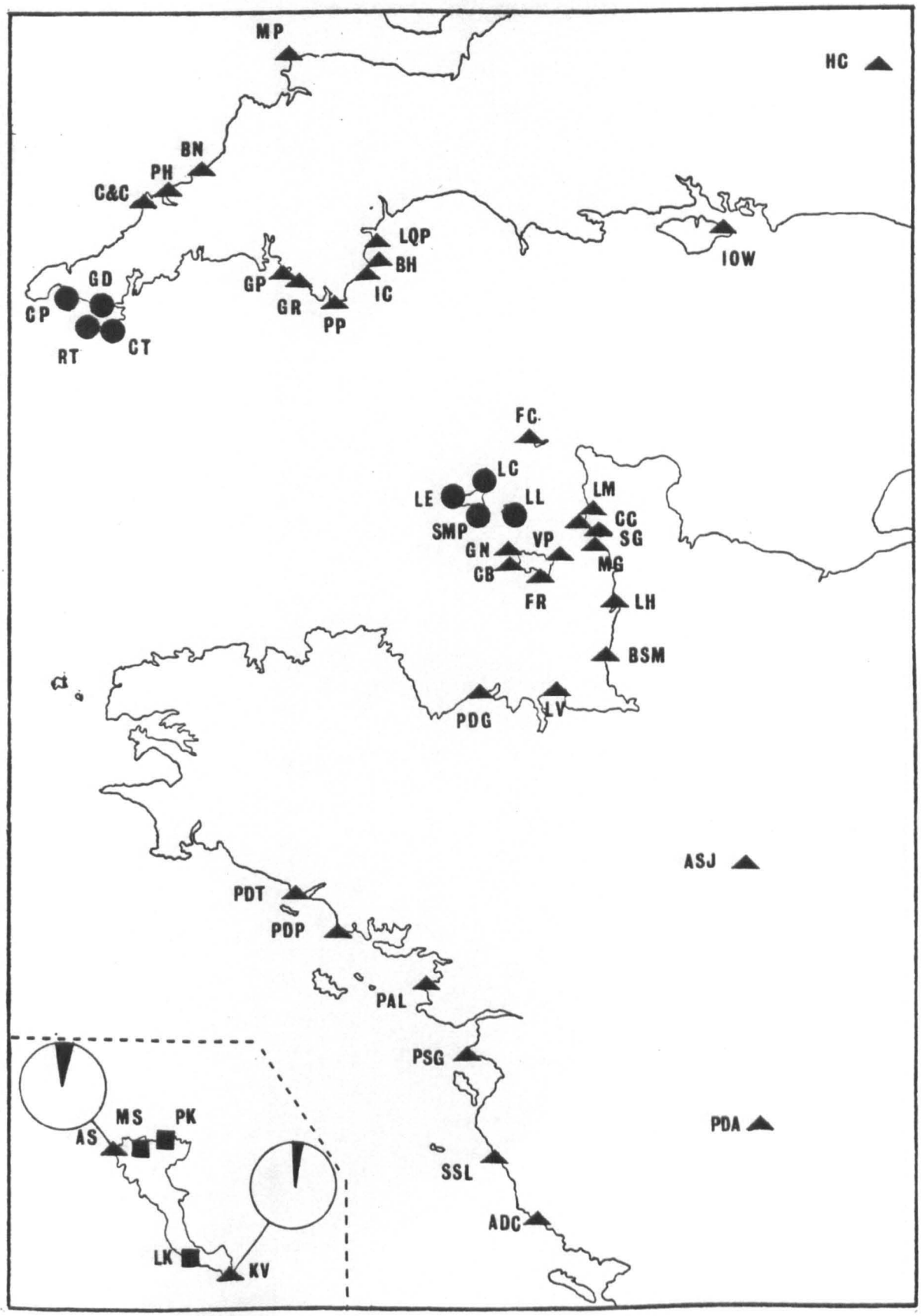
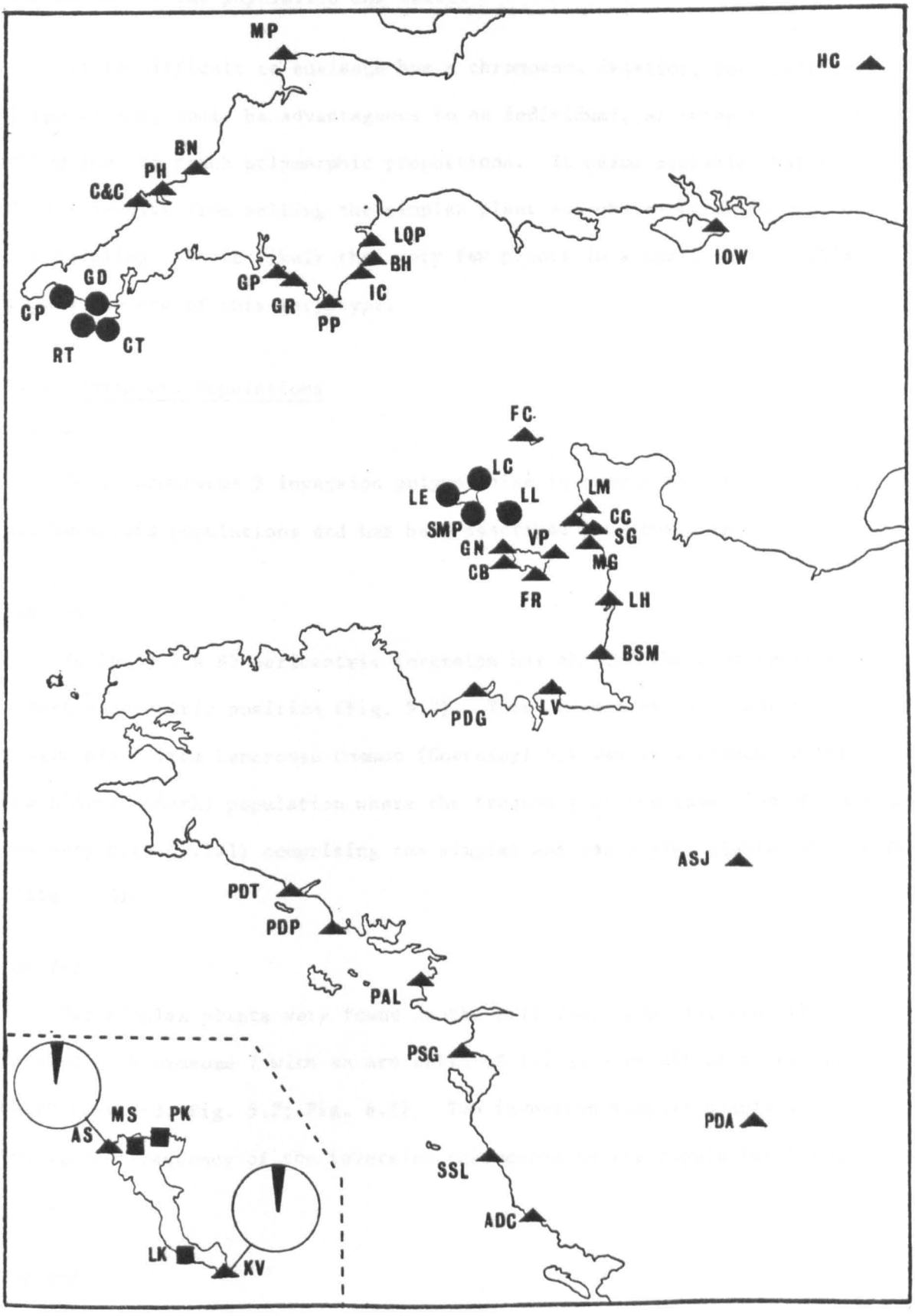


Figure 6.22 Distribution and frequency of Dup 7-1



one duplex (Fig. 6.23; Plate 6.1a and b ). The frequency of the deletion chromosome in the population was 0.023.

It is difficult to envisage how a chromosome deletion, particularly of large extent, could be advantageous to an individual, allowing the deleted chromosome to reach polymorphic proportions. It seems probable that the duplex results from selfing the simplex plant and chance establishment of the seedling. It is likely that very few plants in a small part of this population are of this karyotype.

III. Hexaploid Populations

Inv 3-1

This chromosome 3 inversion polymorphism is common to both tetraploid and hexaploid populations and has been described previously (p.

Inv 3-9

In Inv 3-9 a B3 pericentric inversion has shifted the centromere to a more acrocentric position (Fig. 5.2). This chromosome was present in a single plant from Lancresse Common (Guernsey) but was very common in the Les L'Aches (Sark) population where the frequency of the inversion chromosome was very high (0.121) comprising ten simplex and two duplex plants (Fig. 6.24; Plate 6.2).

Inv 7-3

Two simplex plants were found in the Rill Top, Cornwall, population which carried a chromosome 7 with an arm ratio of 1:2 as a result of a centric shift (Inv 7-3; Fig. 5.2; Fig. 6.1). Two inversion simplex plants were found, the frequency of the inversion chromosome in the population being 0.014.

Dup 3-8

In Dup 3-8 the segment of the long arm distal to the N.O. region in a

Figure 6.23 Distribution and frequency of Del 5-1

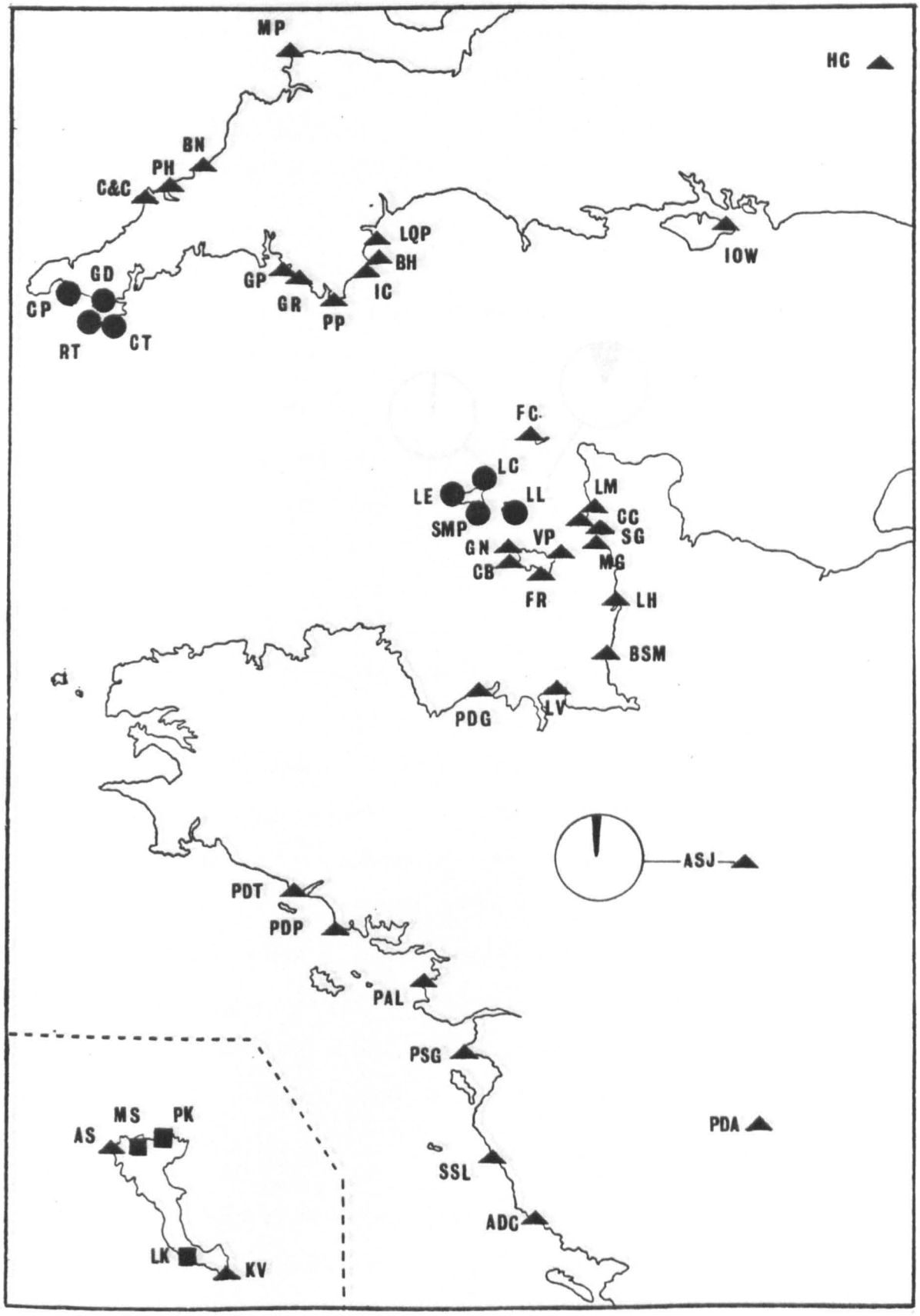
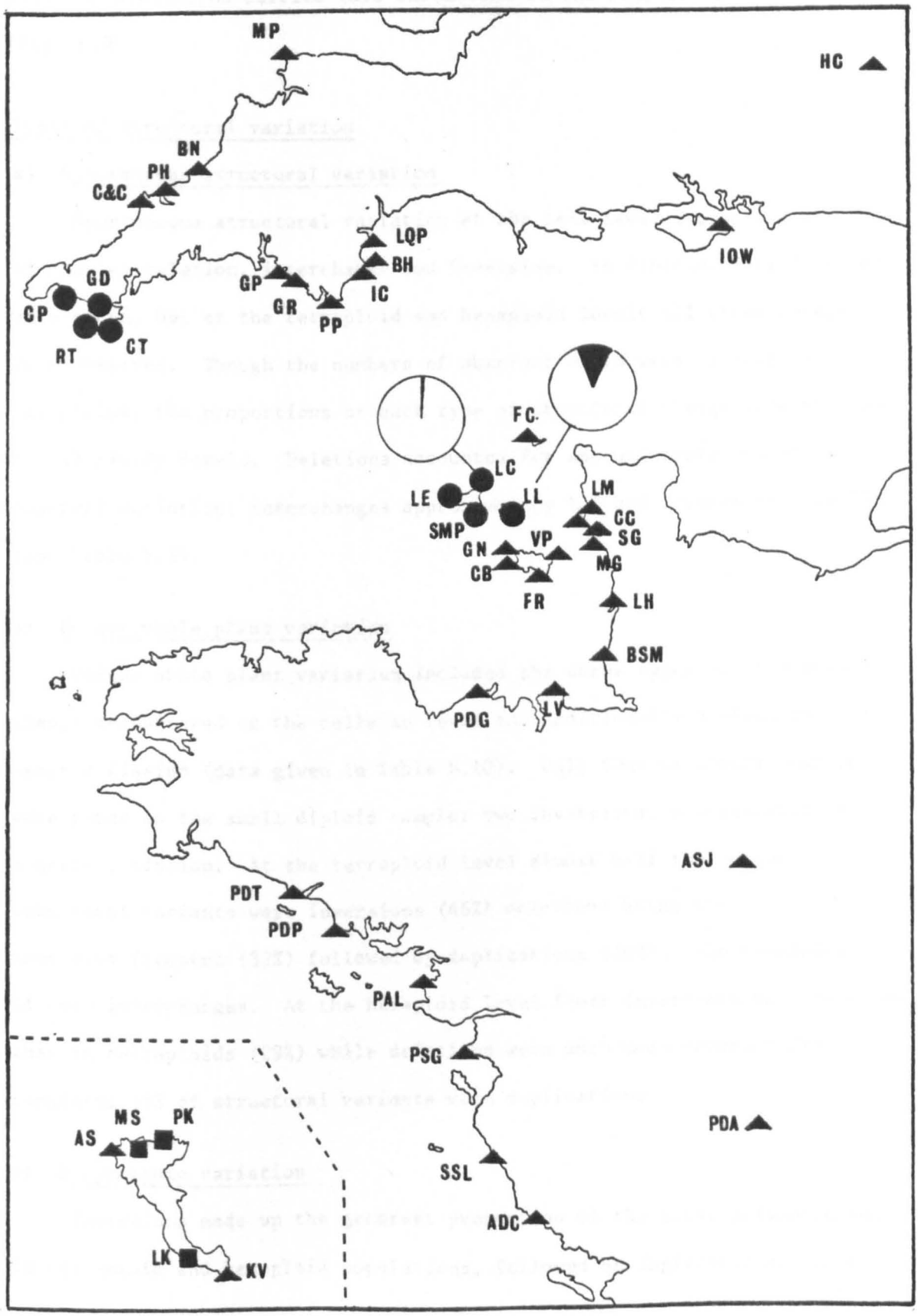


Figure 6.24 Distribution and frequency of Inv 3-9



B3 chromosome was increased by 0.7 μm (Fig. 5.3). Two plants from the Rill Top population carried this chromosome in the simplex condition (Fig. 6.2).

Types of structural variation

a) Spontaneous structural variation

Spontaneous structural variation at the cell level included three types of change: deletion, interchange and inversion. In diploids only deletions were found, but at the tetraploid and hexaploid levels all three categories were observed. Though the numbers of aberrant cells were largest in hexaploids, the proportions of each type of structural change were the same at all ploidy levels. Deletions accounted for approximately 90% of the observed variation, interchanges approximately 10% and inversions only 1% (see Table 5.3).

b) Unique whole plant variation

Unique whole plant variation included the three types of structural change encountered at the cellular level and additionally duplication and centric fission (data given in Table 6.10). Only four structural variants were found in the small diploid sample: two inversions, a duplication and a centric fission. At the tetraploid level almost half the unique structural variants were inversions (46%) deletions being the next most frequent (32%) followed by duplications (20%). The remaining 2% were interchanges. At the hexaploid level fewer inversions were detected than in tetraploids (29%) while deletions were much more common (57%). The remaining 14% of structural variants were duplications.

c) Polymorphic variation

Inversions made up the greatest proportion of the total polymorphisms in tetraploid and hexaploid populations, followed by duplications. Only

Table 6.10 Summary of whole plant structural variation in diploids, autotetraploids and autoallohexaploids of S. autumnalis. a - number of variants. b - number of individuals with that variant.

| Ploidy level | DELETION | | | | | | INVERSION | | | | | | INTER-CHANGE | | DUPLICATION | | | | | | TOTAL VARIANTS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unique | | Polym. | | Total | | Unique | | Polym. | | Total | | Unique | | Unique | | Polym. | | Total | | Unique | | Polym. | | Total | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| 2x | | | | | | | | | | | | | | | | | | | | | | | | | | |
| (81 plants) | - | - | - | - | - | - | 2 | 2 | 1 | 2 | 3 | 4 | - | - | 1 | 1 | 4* | 30 | 5 | 31 | 4+ | 3 | 5 | 30 | 9 | 31 |
| | | | | | | | 2.5% | 2.5% | 1.2% | 2.5% | 3.7% | 4.9% | | | 1.2% | 1.2% | 1.9% | 37.0% | 6.2% | 38.2% | 4.9% | 3.7% | 6.2% | 37.0% | 11.1% | 38.3% |
| 4x | | | | | | | | | | | | | | | | | | | | | | | | | | |
| (1163 plants) | 27 | 21 | 1 | 2 | 28 | 23 | 39 | 31 | 19+ | 364 | 58 | 390 | 3 | 3 | 17 | 13 | 18* | 146 | 35 | 157 | 86 | 37 | 38 | 466 | 124 | 503 |
| | 2.3% | 1.8% | 0.1% | 0.2% | 2.4% | 2.0% | 3.4% | 2.7% | 1.6% | 31.3% | 5.0% | 33.5% | 0.3% | 0.3% | 1.5% | 1.1% | 1.5% | 12.6% | 3.0% | 13.5% | 7.4% | 3.2% | 3.3% | 40.1% | 10.7% | 43.3% |
| 6x | | | | | | | | | | | | | | | | | | | | | | | | | | |
| (246 plants) | 16 | 16 | - | - | 16 | 16 | 8 | 6 | 3** | 84 | 11 | 89 | - | - | 3 | 3 | 1 | 2 | 4 | 3 | 27 | 13 | 4 | 86 | 31 | 99 |
| | 6.5% | 6.5% | | | 6.5% | 6.5% | 3.3% | 2.4% | 1.2% | 34.1% | 4.5% | 36.2% | | | 1.2% | 1.2% | 0.4% | 0.8% | 1.6% | 1.2% | 10.9% | 5.3% | 1.6% | 35.0% | 12.6% | 40.2% |
| Total (1490) | 43 | 37 | 1 | 2 | 44 | 39 | 49 | 39 | 22 | 450 | 71 | 483 | 3 | 3 | 21 | 17 | 22 | 176 | 43 | 191 | 117 | 53 | 47 | 582 | 161 | 633 |
| | 2.9% | 2.5% | 0.1% | 0.1% | 3.0% | 2.6% | 3.3% | 2.6% | 1.5% | 30.2% | 4.8% | 32.4% | 0.2% | 0.2% | 1.4% | 1.1% | 1.5% | 11.8% | 2.9% | 12.8% | 7.9% | 3.6% | 3.2% | 39.1% | 10.8% | 42.5% |

+ Includes one plant with a centric fission

* Includes two polymorphisms common to 2x and 4x

** Includes one polymorphism common to 4x and 6x

one deletion polymorphism was found (tetraploid) affecting only two plants in a single population. By contrast, four of the five polymorphisms detected in diploids, were duplications.

## The extent of structural variation in populations

### a) Diploid populations

Population PK contained the most structural variants with 53% of plants non-standard. Most of the variants were polymorphic with three distinct polymorphisms in this population (Table 5.6). On average only 59% of diploids were standard and about 3% of all chromosomes were structurally variant.

### b) Tetraploid populations

Tetraploid populations contained 55% of structurally and numerically standard plants (Table 5.6). Amongst the 35 populations the overall level of structural variants including non-polymorphic and polymorphic variation ranged from 11% in the Isle of Wight (IOW) population to an amazing 87% at Verclut Point (VP). High values for the overall frequency of structural variation are in general associated with a large number of polymorphic individuals. The level of unique variation averages 3% and in populations ranges from 0% to 13% (SSL). The proportion of structurally-variant chromosomes in tetraploids averaged 2% with a maximum of 6% in the AS (Corfu) population. All 35 populations had at least one polymorphism (Fig. 6.25) and the largest number of polymorphisms in a single population was 7 in the Aghios Stephanos (AS) population from Corfu. Remarkably only 23 plants were scored from this location.

### c) Hexaploid populations

Hexaploid populations on average contained only 48% of structurally and numerically normal plants, 6% lower than tetraploids and 11% lower than diploids (Table 5.6). In 8 hexaploid populations the overall level of

structural variation, including non-polymorphic and polymorphic, was 40% and varied between 6% (GD) and 71% (LC). Polymorphic variation again accounted for the bulk of the variation although no polymorphisms were detected in CP and GD. The overall level of unique variation (5%) was rather higher than that of tetraploids (3%). The proportion of structurally variant chromosomes was, however, lower at 1% and population values ranged from 0.2% (GD) to 2% (LE).

The general trends of chromosomal variation with respect to ploidy level are summarised below. Numerical variation increases in frequency from 1% in diploids to 13% in tetraploids and 20% in hexaploids. In hexaploids this is associated with a high proportion of numerically variable plants, a phenomenon almost absent in tetraploids and diploids.

The overall level of structural variation remains fairly constant between the ploidy levels at around 40% though the level of unique structural variation increases from 2% in diploids to 5% in hexaploids. The level of polymorphic variation shows no trend and is highest in the tetraploids. The proportion of structurally-variant chromosomes decreases with ploidy level from 3% in diploids to 1% in hexaploids with tetraploids intermediate at 2%.

It must be borne in mind that the figures given here for the incidence of structural variation in this polyploid complex are minimum estimates. Symmetrical inversions and exchanges and most paracentric inversions will not have been detected by mitotic screening. Meiotic analysis of a random sample of plants should be attempted to estimate the frequency of these types of structural variation.

## Chromosome variation and ploidy level

Non-polymorphic chromosome variation increased with increasing ploidy level from 4.9 variants per 100 plants in diploids to 10.9 variants per 100 plants in hexaploids with tetraploids intermediate at 7.4 (Table 6.10). However, on the basis of the number of chromosomes present the order is reversed: the hexaploids are the least variable having 2.6 variants per 100 plants per diploid complement, the tetraploids 3.7 and the diploids 4.9 respectively (Table 6.11).

With polymorphic variation the frequency of variant individuals per 100 plants is highest in diploids (6.2) and lowest in hexaploids (1.6) again with tetraploids intermediate at 3.3 variants per 100 plants. When the number of chromosomes is taken into account, the decrease in variation with increased ploidy level is more dramatic, the diploids having ten times more polymorphic variants than hexaploids.

## A and B genome changes in hexaploids

Fourteen spontaneous single cell structural changes in hexaploid plants affected B genome chromosomes while 12 affected the A genome. If structural changes are distributed randomly between the 42 chromosomes according to length then B genome changes should be 1.39 times as frequent as A genome changes. The difference from expected is not significant ($\chi^2 = 0.198$, $P > 0.5$; Table 6.12).

However, when considering whole plant variation in hexaploids the B genome is much more affected by structural change than the A genome ($\chi^2 = 10.035$, $P < 0.01$; Table 6.12).

Table 6.11 Comparison of the numbers of variants per diploid complement in plants of S. autumnalis (per 100 plants).

| Ploidy level | Deletions (unique) | Inversions (unique) | Duplications (unique) | Total variants | | |
|---|---|---|---|---|---|---|
| | | | | Unique | Polymorphic | Total |
| 2x | - | 2.5 | 1.2 | 4.9 | 6.2 | 11.1 |
| 4x | 1.2 | 1.7 | 0.8 | 3.7 | 1.6 | 5.4 |
| 6x | 2.2 | 1.1 | 0.4 | 2.6 | 0.5 | 4.2 |

Table 6.12 The numbers of spontaneous and whole plant structural chromosome variants in the A and B genomes of autoallo-hexaploid plants of S. autumnalis. (Expected numbers calculated on the basis of genome mitotic length)

| | A genome | B genome | Total | $\chi^2$ | P |
|---|---|---|---|---|---|
| No. of spontaneous (cellular) variants | 12(10.88) | 14(15.12) | 26 | 0.198 | >0.5 |
| No. of whole plant variants | 4(12.56) | 26(17.44) | 30 | 10.035 | <0.01 |

# Discussion

## Whole plant structural variation

Five different types of non-polymorphic structural variation have been detected in natural populations of Scilla autumnalis: deletion, inversion, interchange, duplication and centric fission. By contrast only duplications and inversions reach polymorphic proportions in this species. Details of the frequency of variants are given in Table 6.10.

Although the mitotic and meiotic consequences of such chromosomal rearrangements have been well documented (Darlington, 1965; John and Lewis, 1965; Lewis and John, 1963; Sybenga, 1975) the frequencies of structural variants in natural populations of plants have seldom been established. Exceptions among plants are the surveys of the Aloineae by Brandham (1976), Allium schoenoprasum by Bougourd (1977) and Crepis capillaris by Edgar (1981) (Table 6.13).

### I. Deletion

A total of 43 deletions (2.9% of plants) were found in the sample of 1490 plants at diploid, tetraploid and hexaploid levels of ploidy, affecting 37 individuals (2.5%). Remarkably, several individuals carry more than one deletion. Similarly in the Aloineae 1.1% of individuals with deletions were found and all of these were polyploids (Brandham, 1977; Table 6.13). In Scilla no deletions were found in diploids while the frequency of deletion heterozygotes was 1.8% in tetraploids and 6.5% in autoallohexaploids. No deletion heterozygotes were found in diploid Allium schoenoprasum in a sample of 1239 plants (Bougourd, 1977; Table 6.13). The absence of deletions in diploid plants suggests that, as in animals such as Drosophila, deletions are lethal in diploid plants, although they may be buffered in polyploids. Selection against deletions in diploids probably occurs in the gametophyte.

Table 6.13 The frequency of structural chromosome variation in four plant species or groups

| Species | Author | Frequency (%) of individuals | | | | Total plants |
|---|---|---|---|---|---|---|
| | | Deletion | Pericentric inversion | Duplication | Interchange | |
| *Scilla autumnalis* | This thesis (unique) | 2.5 | 2.6 | 1.1 | 0 | 1490 |
| | (total) | 2.6 | 32.4 | 12.8 | 0 | |
| *Allium schoenprasum* | Bougourd (1977) | 0 | 0.2 | 0 | 1.1 | 1239 |
| *Aloineae* | Brandham (1976) | 1.1 | 0.4 | 0.04 | 9.8 | 2234 |
| *Crepis capillaris* | Edgar (1981) | 0 | 1.9 | 0 | 1.1 | 262 |

Deletions in S. autumnalis are approximately twice as frequent per diploid complement in hexaploids (2.2%) as in tetraploid plants (1.2%). This increase is unlikely to be solely brought about by additional chromosomal buffering in the hexaploid and suggests that the hexaploids are more unstable with a higher rate of deletion. This has been shown to be the case for spontaneous deletions at the cellular level (Chapter 5).

Deletions, and spontaneous chromosomal changes in general, which affect individuals rather than cells must either pass through the sexual cycle or be generated at meiosis or during the haplophase. In many plants the levels of spontaneous chromosome breakage are higher at meiosis and pollen grain mitosis than in somatic mitoses. In Najas marina, for example, the frequency of aberrant cell divisions in root tip mitosis was 0.7%, in meiosis 2.6-5.1% and at pollen grain mitosis 2.1% (Viinikka, 1977; Viinikka et al, 1978; Viinikka and Kotimaki, 1979).

In general, the frequency of chromosomal aberrations appears to vary according to the physiological state of the cell and the nature of the tissue. In Trillium, spontaneous breakage occurs in the ovular tissue but almost never in the root tips (Kurabayashi, 1954). It is known that polyploids are able to tolerate structural hybridity and other disturbances better than diploids (Sears, 1944). Brandham (1976), in a survey of the frequency of structural change in gametes from normal plants which survived to form viable progeny, cites figures of 0.07% for haploid gametes carrying deletions and 3.15% for diploid gametes.

Deletions in the Aloineae, reported by Brandham (1971, 1977), include a large deletion in a cultivar of Haworthia resendeae which is thought to represent a single clone. Other deletions in polyploid species of Haworthia occur with a frequency of over 2% (Brandham, 1976). In Scilla autumnalis

no vegetative spread occurs and deletions must, therefore, be transmitted sexually.

II. Inversion

In S. autumnalis inversions (centric shifts) of all seven chromosomes have been detected as well as nucleolar shifts in chromosome B4. Unequivocal proof of inversion, however, requires meiotic analysis. Inversion heterozygosity can only be definitely demonstrated by the presence of inversion loops at zygotene and pachytene. Inversion loops, however, have been demonstrated in only a few cases e.g. Zea mays (McClintock, 1931; Morgan, 1950) and Allium thunbergii (Watanabe and Noda, 1974). Reverse-loop pairing does not always occur in inversions particularly where the inverted segment is short (McClintock, 1933). White (1962) has consistently failed to find reverse loops for presumptive pericentric inversions in grasshopper chromosomes. Indeed, Fletcher and Hewitt (1978) have demonstrated straight non-homologous pairing in a Keyacris scurra centric shift by electron microscopy.

Centric shifts observed at mitosis are usually, but not always, produced as a consequence of inversion. In Haplopappus gracilis (Jackson, 1973) a centric transposition race has been found in which at least 3 breaks are implicated. Centric shifts have been shown in Haworthia by Brandham (1969) to result in E-type bridges. Complex chromosome breakage producing centric shifts has also been demonstrated in Trimerotropis sparsa by White (1951).

In Scilla autumnalis centric shifts and nucleolar-organiser shifts will, for ease of discussion, be referred to as pericentric and paracentric inversions respectively although meiotic analysis is clearly required.

49 inversions affecting whole plants were found in Scilla autumnalis, which represents a frequency of 3.3%. All three ploidy levels were

represented, the frequency of both inversions, and individuals carrying them, varying little between the cytological races. In Allium schoenoprasum, a diploid, the frequency of individuals with spontaneous pericentric inversions was 0.2% (Bougourd, 1977) only one tenth the frequency of diploid S. autumnalis (though this latter figure includes both pericentric and paracentric chromosome 3 inversions). In the Aloineae the frequency of pericentric inversion hybridity was 0.4% which included both polymorphic and non-polymorphic inversions (Brandham, 1976). In Crepis the frequency of inversion heterozygosity was 1.1% (Edgar, 1981). If polymorphic inversions in Scilla are taken into account a frequency of 32% of plants carrying inversion chromosomes is obtained. This figure would presumably be still higher if paracentric inversions for chromosomes other than the N.O. chromosome could be detected. Interestingly, paracentric inversions in the Aloineae are much commoner than pericentric inversions (Brandham, 1976). In the N.O. chromosome of Scilla both pericentric and paracentric inversions which involve the N.O. region can be detected (see Fig. 5.2). Pericentric inversions in this chromosome were approximately twice as frequent as paracentric inversions which included the N.O. region. A third class of inversion in this chromosome was distinguished (complex inversion) in which the positions of both centromere and N.O. region were shifted. Four such variants were found and at least three break points are required for each. It is probable that two successive inversion events have been involved in their origin.

In humans the frequency of inversions has been shown to be low (less than 0.1%) and even in high risk populations attending infertility clinics inversions occur only with a frequency of 0.16% (Court-Brown et al, 1966; Court-Brown, 1967).

Polymorphic inversions

Pericentric inversion polymorphisms have been detected in all B genome chromosomes with the exception of B4, together with paracentric inversion polymorphisms involving chromosome 3, the N.O. chromosome (Table 5.7). A total of 22 inversion polymorphisms have been identified and, discounting paracentric inversions, almost half of all polymorphic inversions affected the N.O. chromosome.

Non-random distribution of inversions between chromosomes has been shown for certain Drosophila species. In D. repleta (Wasserman, 1963) and D. euronotus (Stalker, 1964) there is a marked concentration of inversions in chromosome 2, while in D. pseudoobscura and D. persimilis most inversions are on chromosome 3 (Epling, Mitchell and Mattoni, 1955). Two hypotheses have been advanced to account for the non-random distribution of inversions. A mechanical hypothesis was presented by Novitski (1946) who suggests that, because of the torsional stress of reverse loop formation in inversion heterozygotes, one inversion would tend to lead to the formation of others with closely adjacent break points. There is some evidence in support of this hypothesis (Bernstein and Goldschmidt, 1961; Rothfels and Fairlie, 1957). In the co-adaptation hypothesis of Wasserman (1963) the unequal distribution of inversions is brought about by the action of selection on gene blocks present on particular chromosomes.

In Scilla, where the number of different inversion polymorphisms affecting a particular chromosome in a single population is small, the torsional hypothesis is unlikely to be applicable. It seems, however, that the N.O. chromosome is particularly susceptible to inversion and breakage in general (see below). Selection may well act to amplify any inherent non-randomness. Clearly a role for selection is implicated in the behaviour

of B4 which is seldom involved in inversion, deletion or duplication.

Both pericentric and paracentric inversion polymorphisms have been described in a large number of organisms. The majority of studies which have been carried out on chromosomal polymorphisms in natural populations have been concerned with Dipterans and in particular Drosophila. The superb analytical powers of salivary gland studies (Zeta Karyology of White, 1978) have shown that paracentric inversions are very common in D. willistoni (Da Cunha, Burla and Dobzhansky, 1950; Dobzhansky, 1957), D. subobscura (Goldschmidt, 1956; Prevosti, 1964) and D. pseudoobscura (Dobzhansky and Sturtevant, 1938). Stone (1962) estimates that 42 species of Drosophila contain about 592 polymorphic paracentric inversions. Paracentric inversion polymorphisms have been found in other dipterans such as Simulium in which Landau (1962) identified 83 different inversions, and other animal species such as the Grasshopper, Boonacris alticola (Haines et al, 1978) and the newt Notophthalamus viridescens (Hartley and Callan, 1977).

Dobzansky distinguished in Drosophila between flexible and rigid polymorphisms. By flexible polymorphisms he included those in which the frequency equilibria of the polymorphism vary with latitude, altitude, season or temperature. Inv 3-1 in Scilla appears to be clinal and falls into this category as probably would some of the other inversion polymorphisms in Scilla if the controlling element(s) of the environment could be identified. Polymorphisms of this type are characteristic of many of the endemic Drosophila species such as pseudoobscura, persimilis and robusta. Rigid polymorphisms are those which do not follow such ecogeographical gradients. Geographically widespread Drosophila species such as D. repleta tend to show this type of polymorphism. None of the S. autumnalis polymorphisms seem to be of the rigid type.

Pericentric inversions are less well known than paracentric inversions. Perhaps the best known instances of pericentric inversion polymorphisms come from the grasshoppers particularly the morabine and trimerotropine grasshoppers such as Keyacris scurra (White, 1961) and Trimerotropis pallidipennis (Vaio et al, 1979). Pericentric inversion polymorphisms have also been found in other animals such as the rodents Mastomys natalensis (Matthey, 1966) and Rattus rattus (Yosida, Nakumura and Fakayo, 1965). In contrast to paracentric inversions, pericentric inversion polymorphisms are extremely unusual in Drosophila (Roberts, 1976).

Inversions in plants are remarkably rare and inversion polymorphisms almost unknown. One exception is Haworthia reinwardtii var. chalumnensis in which Brandham (1974) found two pericentric inversions which attained polymorphic proportions. ~~Both inversions were present in all plants examined.~~ Pericentric inversion polymorphisms have also been described in Leontodon hispidus and L. autumnalis (Finch, 1967) and Crepis capillaris (Edgar, 1981). However, there is no parallel in the plant kingdom to the unprecedented frequency and distribution of inversion polymorphisms in Scilla autumnalis. Simonsen (1973) attributed a high incidence of anaphase-I bridge and fragment formation in diploid and tetraploid Lolium perenne to paracentric inversion heterozygosity but an alternative and perhaps more likely explanation is U-type exchange (Lewis and John, 1966).

The genetic consequence of inversion and the maintenance of polymorphisms

Homologous pairing at meiosis in an inversion homozygote (or duplex in the case of tetraploid Scilla autumnalis) may present no problems but in an inversion heterozygote (or simplex) maximum homologous pairing requires the formation of a reverse loop at zygotene.

Chiasmata within reverse loops lead to secondary structural changes and in both pericentric and paracentric inversions duplication-deficiency

chromatids are produced by single crossovers and 3 or 4-strand double crossing over. With paracentric inversions these are associated with the production of dicentric chromatid bridges and centric fragments. The fate of the dicentric bridges depends on the organism: unbalanced gametes may be produced, breakage-fusion-bridge cycles may be initiated, or they may be selectively eliminated from forming functional gametes (John and Lewis, 1965). The genes within the inversion can be inherited as a linked block undisturbed by recombination although the organism may suffer a decline in fertility as a result.

The property of inversions to act as cross-over suppressors is absolute in many grasshoppers since chiasmata do not form in pericentrically inverted segments in heterozygotes (White, 1973). Reverse loops may well not be found (Coleman, 1948) and pairing has been shown to be 'straight' in the inverted segment of inversion heterozygotes of Keyacris scurra (Fletcher and Hewitt, 1978). In view of the frequent and widespread nature of inversions in Scilla and the genetic load imposed by chiasmata within reverse loops especially in diploids, it is possible that pairing in the inverted segments in Scilla is also straight, though as yet there is no evidence for this. By contrast, Noda (1974) has reported a pericentric inversion in Scilla scilloides with a high frequency of chiasmata within the inversion. The presence of relatively infertile inversion heterozygotes was attributed to the effective vegetative spread of this species.

An additional effect of inversions is that they may alter the chiasma frequency in the unmodified elements of the complement, an effect well known in Drosphila (Schultz and Redfield, 1951; Suzuki, 1963). By contrast to such interchromosomal effects, pericentric inversions in species of Trimerotropis (White and Morley, 1955) suppress chiasma formation in the inverted segment but increase chiasma formation in the regions distal to

the rearrangement. This has the intriguing consequence of increasing linkage of genes on one part of the chromosome but loosening linkage amongst genes on another part of the same chromosome.

III. Interchange

Interchanges are the least common of all structural rearrangements found in natural populations and interchange systems are known in relatively few plants and in still fewer animals (John, 1976).

In Scilla autumnalis only three interchange heterozygotes were detected in a sample of 1400 plants, all three being in tetraploids. No polymorphic interchanges were found. However, for definitive identification of interchanges meiotic analysis is required. The overall frequency of interchange hybridity then was only 0.2% making it the least frequent of the four main classes of structural rearrangement identified in Scilla. Interestingly, the converse was true both for Allium schoenoprasum and the Aloineae. In Allium (Bougourd, 1977) the frequency of interchange was 1.1%, in Crepis capillaris 1.1% (Edgar, 1981) while in the Aloineae it was a remarkable 9.8% (Brandham, 1976). In the latter case, most interchanges occurred in polyploid species and several were polymorphic (Brandham, 1974).

In newborn humans the frequency of both balanced (Jacobs et al, 1974) and unbalanced (Carr and Gedeon, 1977) translocation is 0.2%. Translocations are produced with a much higher frequency than this since 2.4% of all (chromosomally) abnormal abortuses contain unbalanced translocations (Carr and Gedeon, 1977).

IV. Duplications

Duplications or supernumerary chromosome segments were found in 12.8% of individuals in Scilla autumnalis, including both unique and polymorphic duplications. Forty-two separate duplications were identified, over half

of which were polymorphic. Whole plant unique duplications were found in 1.1% of individuals. Little variation in the incidence of duplications is found between the three ploidy levels both in respect of the number of individuals carrying duplications and the number of different duplications.

Supernumerary segments are well known in Orthopterans (Hewitt and John, 1968; John, 1973). However, chromosomal duplications are very rare in plants. In a survey of 2234 Aloineae plants (Brandham, 1976) a single individual with a duplication was found (0.04%). In surveys of 1239 Allium schoenoprasum plants (Bougourd, 1977) and 262 plants of Crepis capillaris (Edgar, 1981) no individuals with duplications were recorded. Few polymorphic supernumerary systems have been recorded in plants although the extensive variation in heterochromatin pattern in species such as Scilla sibirica (Vosa, 1973) may play a similar role in the genetic system.

Dobzhansky (1941) and Metz (1947) have emphasised the importance of duplications as the only method, apart from polyploidy, by which the amount of genetic material in the germ plasm of an organism can be increased. Ohno (1970) has further explored the role of duplication as an active evolutionary mechanism particularly in the higher vertebrates.

Polymorphic duplications

Twenty two of the total of 43 duplications attained polymorphic proportions. Most polymorphisms are restricted to one or a few populations (Table 6.14) though one duplication was found in 7 populations. The frequency of different duplication polymorphisms was highest in the diploid populations (1.9 duplications per 100 plants) the hexaploid populations exhibiting the lowest frequency (0.4%) with only one duplication found. The numbers of individuals carrying polymorphic duplications in diploids amounted to 37% and was 12.6% in tetraploids and 0.8% in hexaploids.

Table 6.14 Polymorphic variants and the numbers of populations with that variant

| Polymorphic variants | No. of populations with each chromosome variant | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 6 | 7 | 12 | 34 |
| Inversion | 16 | 3 | 1 | - | - | - | 1 | 1 |
| Duplication | 10 | 5 | 3 | 1 | 1 | 1 | - | - |
| Deletion | 1 | - | - | - | - | - | - | - |
| Total variants | 27 | 8 | 4 | 1 | 1 | 1 | 1 | 1 |

Polymorphisms for duplicated chromosome segments have previously been recorded in plants only in the diploid Leopoldia weissii in which two populations from a single island in the Cyclades group, Greece, contained individuals with a supernumerary segment on the short arm of chromosome 4 (Bentzer, 1972).

In grasshoppers, supernumerary segment systems are particularly common (Nur, 1961; John and Hewitt, 1966; Shaw, 1970). These supernumerary segments are in all instances heterochromatic (White, 1973). In Scilla, by contrast, none of the duplications appeared heterochromatic in somatic mitoses. However, the duplicated arm of Dup 2-1 appeared wider at mitotic C-metaphase and was clearly heterochromatic at first meiotic prophase (Plate 6.4 b-f). Other duplications such as Dup 1-1 and Dup 1-6 in S. autumnalis are not heterochromatic at meiosis and no case of increased width of duplications similar to Dup 2-1 were found. Also there is no evidence of chromocentres in interphase nuclei.

In grasshoppers, it may be the heterochromatic nature of supernumerary segment systems which is responsible for maintaining the polymorphism. Supernumerary segments have been shown in Chorthippus parallelus and other species to cause an increase in mean chiasma frequency (Hewitt and John, 1968; John, 1973; John and Hewitt, 1969; Westerman, 1969) as has supernumerary heterochromatin not integrated into the genome in the form of B-chromosomes (John and Hewitt, 1965a and b; Jones, 1975). In the case of the heterochromatic Dup 2-1 in S. autumnalis the chiasma frequency of the hetermorphic bivalent was lowered since chiasmata rarely occurred in the short arm. The mean chiasma frequency per PMC, however, was higher in the duplication heterozygote than in two normal plants though meiotic data from many more plants is required to establish whether this is a general effect. In view of the high frequency of variant chromosomes in some of the duplication polymorphisms (Dup 1-1, 1-3 and 5-2 in tetraploids

and Dup 2-1 in diploids) the selective forces which are responsible for the maintenance of the polymorphism must be high.

Duplications can be produced in several ways:-

1. unequal interchange with loss of the smaller interchange product; the reciprocal product would then appear as a deletion.
2. direct duplication from breakage of meiotic chromatid bridges in inversion heterozygotes or after U-type exchange.
3. recovery of unbalanced crossover products from pericentric inversion heterozygotes; the reciprocal again would be recorded as a deletion (Brandham, 1977b).

All these mechanisms give rise to duplication deficiency products which are normally inimical to gametic survival or zygotic development. Indeed Sjödin (1971) demonstrated death of gametes carrying crossover products from pericentric inversion heterozygotes in the diploid Vicia faba.

However, in a tetraploid Aloe hybrid carrying a pericentric inversion both duplication and deletion products resulting from crossovers in reverse loops were transmitted through both pollen and eggs to the progeny (Brandham, 1977b). In tetraploid paracentric inversion heterozygotes chromosomes carrying deletions as a result of broken anaphase bridges were also transmitted in viable gametes (Brandham, 1977a). If the situation in Scilla autumnalis is similar then one would predict that both the number of deletions and the number of duplications affecting a particular chromosome group would be proportional to the number of inversions. Duplications exhibit this relationship as do deletions with the exception of B2 which appears particularly susceptible to deletion (Figs. 6.25 and 6.26). Survival of unbalanced crossover products of inversion heterozygotes, thus, may in part explain the unusual frequency of duplications in S. autumnalis

Figure 6.25 The number of deletions and number of inversions affecting the B genome chromosomes of S. autumnalis (paracentric B3 inversions excluded)

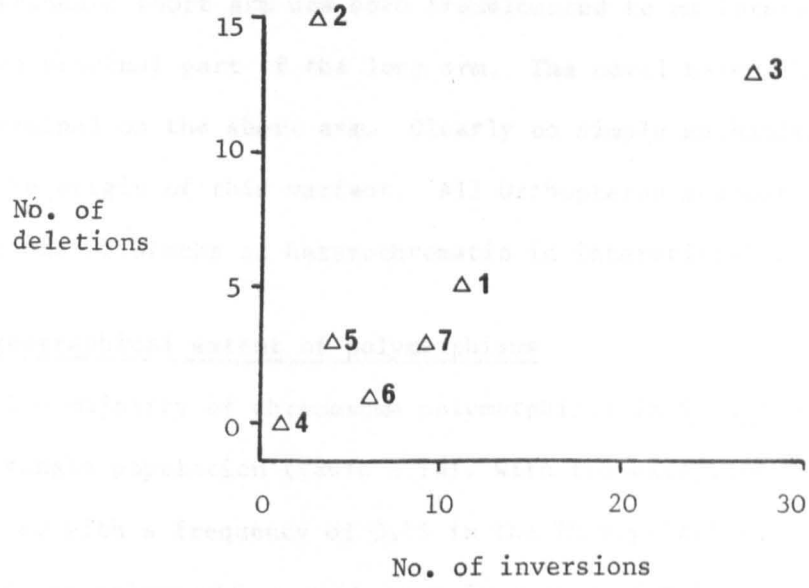
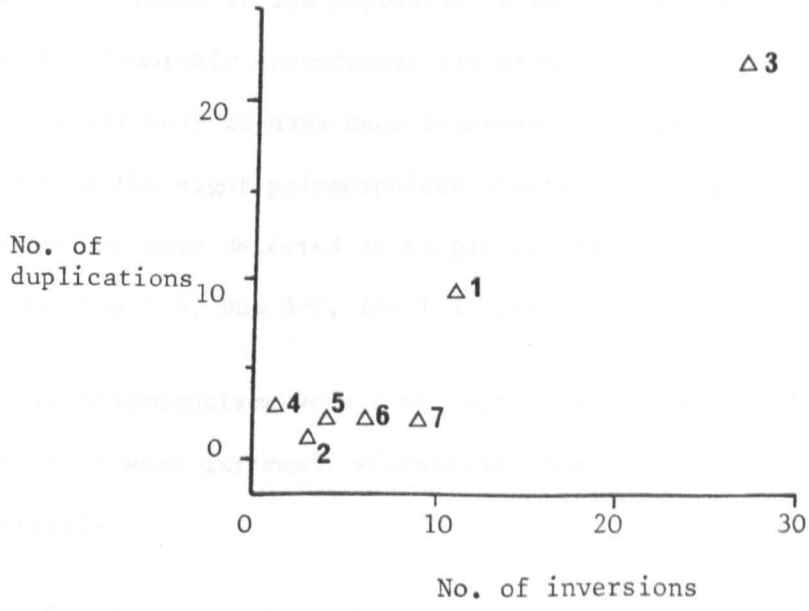



Figure 6.26 The number of duplications and number of inversions affecting the B genome chromosome of S. autumnalis (paracentric B3 inversions excluded)



Meiotic evidence for the structure of the duplication chromosomes in Scilla is only available for the Dup 2-1 variant. Meiotic configurations suggest a complex rearrangement has occurred in which the major part of the standard short arm has been translocated to an interstitial location in the proximal part of the long arm. The novel heterochromatic segment is terminal on the short arm. Clearly no simple mechanism can be invoked for the origin of this variant. All Orthopteran segment systems are simple additions of blocks of heterochromatin in interstitial or terminal positions.

The geographical extent of polymorphisms

The majority of chromosome polymorphisms in S. autumnalis are confined to a single population (Table 6.14). With the exception of Dup 2-1 which occurred with a frequency of 0.15 in the PK population, all single population polymorphisms were at a frequency of 0.05 or less. This situation would be expected for two reasons: (i) a polymorphism exists in a population at a low level either because it is of recent origin or because it is maintained at this level by selection, (ii) if a polymorphism is present in more than one population then, with a limited sample, it is most likely to have been found in the population with the highest frequency. Populations in which polymorphic chromosomes are present in less than 1 in 30 individuals are unlikely to have been detected. We can exemplify this pattern by considering the eight polymorphisms limited to two populations. Four of these polymorphisms were detected as single plants in the lower frequency populations (Dup 1-5, Dup 3-7, Inv 1-4, Inv 3-9).

Four polymorphisms were distributed over three populations and a further four were extremely widespread, found in 6, 7, 12 and 34 populations respectively.

In Scilla autumnalis, then, the general trend in the geographical distribution of chromosomal polymorphisms is that they are present in a

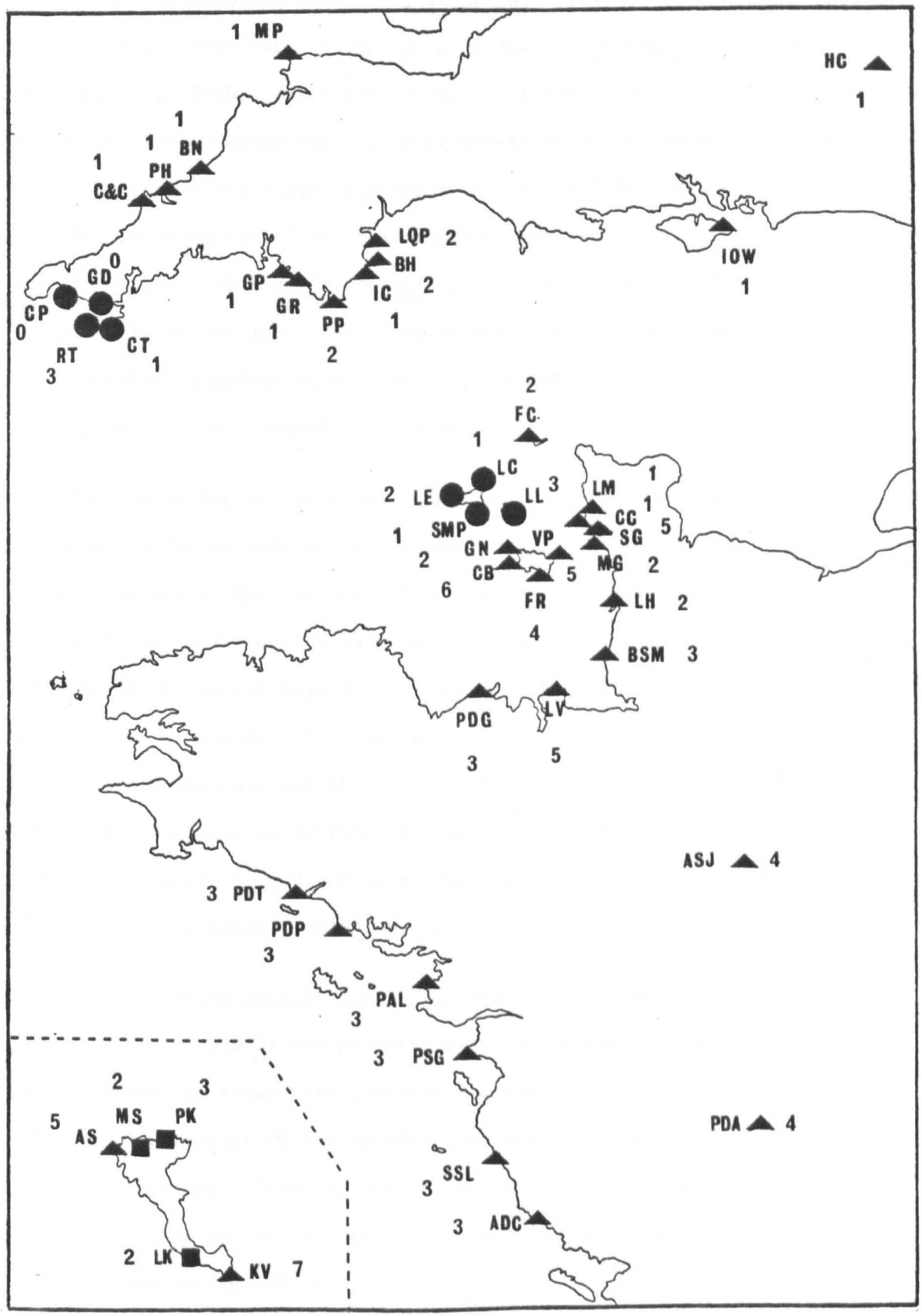
very small number of populations with a very rapid decline in frequency around the epicentre. Thus chromosome polymorphisms are, on the whole, very local. An excellent example of this is Inv 6-1, centred on Jersey and the Cotentin Peninsula, where populations have a high frequency of this chromosome. The chromosome is absent from several populations in Brittany and inland French populations (Fig. 6.14).

Some distributions may be indicative of founder effects and again Inv 6-1 may illustrate this. The variant chromosome is absent from CC and its neighbouring population to the south SG contains only one inversion chromosome in 151 plants. To the north and south, populations LM and BSM have very high frequencies of the inversion chromosome. Possible founder effects are also evident in the extremely widespread Inv 3-1 polymorphism. Four scattered populations did not contain the variant chromosome although these were adjacent to, and surrounded by, areas of high frequency (Fig. 6.10).

Dup 1-1 is unusual in that it is confined to four adjacent populations in South Devon from Long Quarry Point in the north to Prawle Point in the south (Fig.6.15). The frequency of the duplication chromosome is relatively high in each of these populations (0.039 - 0.156). The neighbouring populations to the west, GR and GP, are not polymorphic for Dup 1-1, although they are only about 30 km from the Prawle Point population.

In tetraploid populations the total number of polymorphisms per population varied between 1 and 7 (Fig. 6.27). British populations tended to have fewer polymorphisms than those in France and the Channel Islands. The number of polymorphisms per population shows a significant negative relationship with latitude such that populations in the south of the sample area tend to carry more polymorphisms than those in the north ($t = 6.81$,

Figure 6.27 The numbers of polymorphisms present in populations of S. autumnalis



$P < 0.001$; Fig. 6.28). Particularly notable are the two tetraploid populations from Corfu, AS and KV, which have five and seven polymorphisms respectively. This data is analogous to that in Drosophila willistoni reported by Da Cunha, Burla and Dobzhansky (1950) and Da Cunha and Dobzhansky (1954) where the degree of inversion polymorphism is greatest in the centre of the range (equatorial Brazil) and declines regularly towards the periphery of the distribution. Similar observations have been made by Carson (1959) on D. robusta in the United States. Central populations in Missouri and the Appalachian region are highly heterozygous for inversions while marginal populations in Nebraska, Vermont and Florida are mainly homozygous for the standard gene sequences.

Dobzhansky has argued that the amount of chromosomal polymorphism in these species is related to the diversity of ecological niches occupied by the population. The species will occupy the greatest number of ecological niches of the centre of its range where ecological and climatic factors are optimum and at the edge of its range may be able to exploit only a small number of niches. This may well be the case in Scilla where the populations in Britain are at the extreme northern limit of the distribution. Habitat requirements in Britain are very precise leading to widely separated populations, while in Mediterranean regions such as Corfu the species is distributed more nearly continuously.

An alternative explanation of the Drosophila pattern has been put forward by White (1973) who proposes that such a species may only recently have extended its range into peripheral areas. Not all the chromosomal polymorphisms present in the species then may have reached the newly-occupied territory. Finally, not all species show a decline in polymorphism to the periphery of the distribution, e.g. Trimerotropis gracilis in the United States (White, 1973).

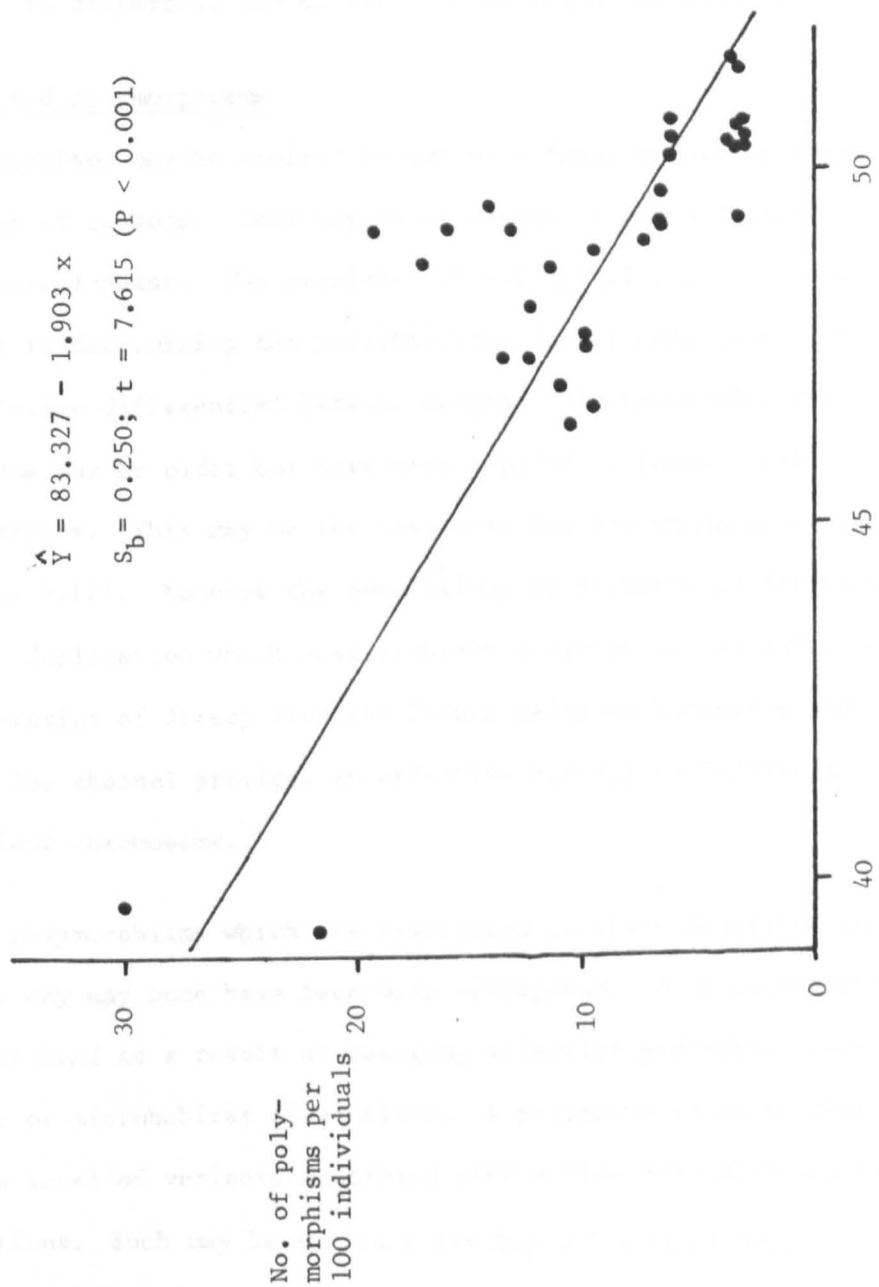



Figure 6.28 Regression of the number of different polymorphisms in populations of S. autumnalis (expressed as the number per 100 individuals) against latitude.

## The geographical origin of polymorphisms

The geographical distribution of a polymorphism is dependent on a) the selective advantage conferred on the individual carrying the variant chromosome; b) the geographical location of the individual when the initial mutation occurred; c) the nature of the subsequent spread of plants carrying the polymorphic chromosome, influenced by the presence and nature of barriers to dispersal, and d) the time at which the mutation arose.

### i) Restricted polymorphisms

Polymorphisms may be limited to one or a small number of populations for a number of reasons. They may be of recent origin and still in an active dispersal phase. The proximity of neighbouring populations will be critical in determining the possibilities for spread, as will the size of the selective differential between morphs. Alternatively, limited polymorphisms may be older but have been impeded in their spread by a physical barrier. This may be the case with Dup 1-3 which is confined to Jersey (Fig. 6.17). None of the populations in Normandy or Brittany contain the duplication which most probably arose on Jersey subsequent to the separation of Jersey from the French mainland between 6 and 7,000 years BP. The channel provided an effective barrier to further movement of the variant chromosome.

Other polymorphisms which are restricted in their distribution at the present day may once have been more widespread. Such polymorphisms may have declined as a result of changing selective pressures occasioned by climatic or microhabitat alterations. A polymorphism in retreat may well leave a low level of variants scattered over a wide area although not in all populations. Such may be the case for Dup 4-1 (Fig. 6.19).

ii) Widespread populations

The term 'widespread' is used here only in relation to the populations of N.W. France and southern Britain. Four widespread polymorphisms occur in this area and in each the two populations furthest apart are separated by at least 200 km. These are Dup 1-2, Dup 4-1, Inv 3-1 and Inv 6-1. These polymorphisms are likely to be older than most of the more restricted ones and it seems likely that they predated, or accompanied, the colonisation of north-western Europe.

Polymorphisms involving more than the chromosomal races

Three polymorphisms were detected which were present in two different chromosomal races. Two duplications Dup 1-5, Fig. 6.3; Dup 1-6; Fig. 6.4) were found in both diploid and tetraploid populations on Corfu and the extensive Inv 3-1 polymorphism was found in both tetraploid and autoallohexaploid populations in north-western Europe. Polymorphisms such as those which cross ploidy levels may either predate the evolution of the complex, or have been transferred by hybridisation.

Inv 3-1

Inv 3-1 was present in 28 of the 35 tetraploid populations and six out of the eight hexaploid populations (Figs.6.9 and 6.10). If the autoallohexaploid evolved from hybridisation between tetraploids and diploids (Chapter 7) then the inversion could have been transferred from tetraploid to hexaploid. This origin, however, appears unlikely (see Chapter 7).

A more likely hypothesis is that the inversion arose in the autoallohexaploid and introgressed into the tetraploid race by hybridisation and back-crossing. The implication of this is that the tetraploid populations in which the inversion is present are relict hexaploid populations. The evolution and present day geographical distribution of Inv 3-1 thus may have

an important bearing on the relationships and evolution of the chromosomal races of Scilla autumnalis (Chapter 7).

Dup 1-5 and Dup 1-6

These polymorphisms, which occur in both diploid and tetraploid races on Corfu, are similarly most likely to have been transferred between races by hybridisation and introgression. Since the two duplication polymorphisms do not extend to all populations on the island it seems probably that the origin of the polymorphisms, and their transference, is recent. A detailed survey of Corfu might be expected to reveal triploids and backcross derivatives if this hypothesis is correct.

The Hardy-Weinberg equilibrium

Although the numbers of individuals in most populations were too low to test whether polymorphisms were in Hardy-Weinberg equilibrium, all those that were so tested were in equilibrium. Also there was no indication of disequilibrium in the low frequency populations. Now, the Hardy-Weinberg equilibrium is based on certain assumptions, some or all of which will be invalid in a real population. The requirements for derivation of the equilibrium are that there is no selection, mutation or migration, the theoretical population being very large and random-mating. Now it is highly unlikely that structural heterozygotes from a clinal polymorphism such as Inv 3-1 will be selectively neutral. The Hardy-Weinberg equilibrium, however, is very robust and deviations from the expected due to any of the causes outlined above must be very large to be detected by a $\chi^2$ analysis. Selective differentials on a chromosome polymorphism are unlikely to be extreme enough to be detectable in a population of 30 plants in a single generation. Though $\chi^2$ analysis was performed on the observed and expected numbers of plants for all the polymorphisms no significant deviations from the H-W proportions were apparent.

Nucleolar-organiser chromosome variation

The nucleolar-organiser chromosome B3 shows more structural variation than any other at the level of whole plant and polymorphic variation. Although 21 out of 112 single cell events involved B3 this is not significantly different from the expectation on the basis of chromosome length. Considering whole plant structural change, the N.O. chromosome was involved in almost half the total numbers of deletions, duplications and inversions (Table 5.7).

Why does the nucleolar-organiser chromosome exhibit such variability? Part of the answer may lie in the fact that the distinctive morphology of this chromosome with its two adjacent markers enables structural changes to be readily detected but there may be a more fundamental reason.

Nucleolar-organiser chromosomes have been found to be unstable in many organisms (Dyer, 1963). In Leopoldia the varying distribution, form and number of satellites in tetraploid and hexaploid populations was thought to indicate frequent small inversions and translocations in the distal chromosome segments (Bentzer 1969, 1972b). Similarly, Von Bothmer (1975) attributes considerable N.O. chromosome variation in Allium bourgaei to differences caused by inversion.

Perhaps the best example of nucleolar chromosome instability parallel to Scilla autumnalis comes from Elymus where extreme instability and polymorphism of this chromosome was shown in three species: diploid E. striatulus, tetraploid E. rechingeri and octoploid E. diae (Heenen and Runemark, 1962, 1972; Heenen, 1977a, 1977b, 1977c). Additional constrictions in the nucleolar-organiser chromosome similar to those in Scilla were found in this genus. Other organisms which have been shown to be particularly susceptible to breakage in the nucleolar chromosome are Allium species (Ved Brat, 1965) and

Tomato (Jain in Ved Brat, 1965) in which 50% of spontaneous structural mutations occurred in the nucleolar chromosome. In Allium schoenoprasum eight out of 13 interchanges involved the N.O. chromosome (Bougourd, 1977).

The high variability in nucleolar-organiser chromosomes might be correlated with the specific structural organisation of these chromosomes. The association of the N.O. chromosomes with the nucleolus may impose physical contraints which increase susceptibility to breakage around the NOR itself. Also, the lability of repetitive DNA has been shown by various authors (Britten and Kohne, 1968; Flamm, 1972; Flavell et al, 1974; Holmquist, 1975; Narayan and Rees, 1976) and it is well documented that repetitive DNA sequences occur at N.O. sites (Birnstiel et al, 1966; Pardue, 1975).

Studies in Vicia faba have indicated a contrary situation. In this species the N.O. chromosome is less affected by radiation-induced interchange than other chromosomes (Evans and Biggar, 1961). Perhaps in this case the nucleolus acts as a physical impediment to exchange between non-homologues.

In Scilla autumnalis the interaction of the nucleolar-organiser chromosomes and nucleolus may be an important factor in influencing both the frequency and distribution of aberrations along the length of the N.O. chromosome. The numbers of B3 deletions are too small to enable within-chromosome comparisons to be made. Twenty-two duplications were recovered and these can be partitioned into those affecting the short arm, centromere-nucleolar-organiser region of the long arm, and distal region of the long arm (Table 6.15). When treated as equal units these three regions have the same number of duplications ($\chi^2_{(2)} = 13.06$, $P < 0.01$). When the length of each segment is taken into account, however, fewer than expected duplications occur in the short arm, the expected number affect the distal segment of the long arm, but many more affect the centromere-NOR

Table 6.15 The numbers of duplications involving the three regions of the B3 nucleolar-organiser chromosome. Expected values are calculated on the basis of equal numbers in each region (a) and in proportion to chromosome length (b).

| Chromosome region | length (μm) | No. of duplications observed | expected a | expected b |
|---|---|---|---|---|
| short arm | 2.36 | 3 | 7.33 | 8.06 |
| long arm { centromere - N.O. | 1.13 | 10 | 7.33 | 3.86 |
| long arm { N.O. - telomere | 2.95 | 9 | 7.33 | 10.08 |
| | | 3 | $\chi^2 = 3.59$ ($P > 0.05$) | $\chi^2 = 13.06$ ($P < 0.001$) |

segment ($\chi^2_{(2)}$ = 13.06, P < 0.01). Evidence from mouse and man indicates that the locations of NORs determined by silver staining may vary (Dev et al, 1977). It has been suggested by Hsu (1979) that this is caused by synapsis and crossing-over between the ribosomal sequences of non-homologous chromosomes which are brought into close contact by association with a common nucleolus. In Homo sapiens anomalies in the short arms of D and G group chromosomes are particularly frequent (Hsu, 1979). Crossing-over between NOR chromosomes while attached to the nucleolus is a possible explanation for the extreme variability of the N.O. chromosome in Scilla autumnalis.

Nucleolar-organiser expression

The only known function of secondary constrictions is the organisation of the nucleolus in the interphase nucleus (Heitz, 1931).

In diploid populations of Scilla autumnalis a secondary constriction was always observable in both B3 chromosomes in all plants.

In tetraploids a maximum of four secondary constrictions (N.O. regions) was observed in most plants. In occasional plants from sixteen tetraploid and three hexaploid populations some B3 chromosomes did not exhibit a nucleolar-organiser region so that in some cases four NORs were apparent and in others only three. (Plate 6.5).
Nine plants were constant in the deviant numbers of NORs, eight showing three secondary constrictions and a single plant only two (Table 6.16).

Variation in N.O. expression is probably a genic phenomenon. In view of the high frequency of structional variation in Scilla autumnalis, however, it is possible that NORs may have been deleted completely in the plants showing constant expression.

Plate 6.5 Variation in nucleolar-organiser expression. A cell with three NORs

Table 6.16 Variation in expression of B genome nucleolar-organiser regions in sixteen autotetraploid and three autoallohexaploid populations of S. autumnalis

| Population | | Number of plants | | | |
|---|---|---|---|---|---|
| | | Constant expression No. of organisers | | | Variable expression No. of organisers |
| | | 4 | 3 | 2 | 3/4 |
| 4x | MP | 26 | - | - | 2 |
| | BN | 30 | - | - | 2* |
| | PH | 29 | - | - | 1 |
| | C+C | 33 | - | - | 1 |
| | GP | 28 | - | - | 1 |
| | GR | 31 | - | - | 1 |
| | IOW | 31 | - | - | 5 |
| | CB | 30 | 1 | - | - |
| | CC | 28 | 1 | - | 1 |
| | SG | 148 | 2 | - | 1 |
| | MG | 29 | - | - | 1 |
| | LH | 26 | 1 | - | - |
| | DDG | 25 | - | - | 1 |
| | PSG | 23 | - | - | 1 |
| | SSL | 30 | - | 1 | - |
| | ADC | 26 | - | - | 2 |
| 6x | GD | 30 | 1 | - | - |
| | LC | 27 | 1 | - | - |
| | LE | 26 | 1 | - | 1 |

*Includes one plant with 2/3

The number of nucleolar-organisers within the chromosome complement is generally characteristic of a species (e.g. Darlington, 1965), but variation in the numbers of N.O. regions expressed has been previously shown in Nigella (Strid, 1969) and in Allium schoenoprasum where extensive NOR variation has been reported (Bougourd, 1977). Doubts about the intra-specific constancy in rDNA gene number were first raised by observations on amphibian lampbrush chromosomes in which the NORs of each half bivalent were of different sizes (Macgregor, 1965; Callan, 1966). Nucleic acid hybridisation experiments have confirmed that the actual amount of rDNA varies between homologues (Macgregor and Kezer, 1971; Hennen et al, 1975). Differences in rDNA content between individuals from the same proportions have also been demonstrated (Miller and Brown, 1969; Macgregor et al. 1977). On purely cytological evidence and without complementary rDNA analysis in S. autumnalis it is impossible to say whether the observed variation in NOR number is due to differences in expression or real differences in rDNA content. However, the variability of NOR numbers between cells of the same plant must result from expression differences.

Nucleolar-organiser suppression

In autoallohexaploid plants of S. autumnalis a maximum of four NORs are observed, all four in the B3 chromosomes. In the allotetraploid (AABB) there are only two NORs again in the B3 chromosome. No secondary constrictions have been observed in A-genome chromosomes at these ploidy levels. In the AA diploid, however, two nucleolar-organiser regions are always present. In AABB and AABBBB hybrids, therefore, the A genome NORs are suppressed by those of the B genome.

Nucleolar-organiser suppression (amphiplasty) was first recorded by Navashin (1934) in Crepis interspecific hybrids and has subsequently been detected

in many groups of plants and animals. In Hypochoeris glabra x radicata hybrids the H. radicata NOR is suppressed by the H. glabra genome (Parker, 1975) and suppression has recently been shown to be attributable to the H. glabra N.O. chromosome itself (Whitehorn, pers. comm.). In interspecific Hordeun hybrids suppression of N.O. constrictions is not always accompanied by a parallel suppression of nucleolus formation (Subrahnanyam and Azad, 1978) thogh this is not the case in other organisms. Work by Maggini et al (1976) on Scilla autumnalis and Urginea maritima showed that there was approximately the same percentage of rDNA in diploid, autotetraploid and autohexaploid plants of both species.

# CHAPTER SEVEN

## THE EVOLUTION OF THE COMPLEX

### Introduction

The epithet Scilla autumnalis refers to a polyploid complex containing diploid, tetraploid and hexaploid levels. Six chromosomal races occur with two distinct genomes involved, referred to as the A and B genomes (Chapter 3; Table 3.1; Fig. 3.1). In this chapter the relationships between these races at the levels of both exophenotype and endophenotype are considered.

1. Diploid AA

This diploid race (2n = 2x = 14) has been found on coastal limestone in a single locality in Portugal (Peniche), 80 km north of Lisbon.

2. Diploid BB

The diploid BB race (2n = 2x = 14) is found at the southern end of the species distribution. It extends from the countries along the north coast of Africa to the Levant and reaches across the Mediterranean to Turkey and Greece and the southern parts of Italy and Spain.

3. Tetraploid BBBB

The autotetraploid race (2n = 4x = 28) has a more northerly distribution and overlaps with the diploid race in Spain, Italy, Greece and the Mediterranean Islands. This race reaches southern England which is the northern limit of the distribution of the complex.

4. Tetraploid AABB

The allotetraploid race (2n = 4x = 28) has been found only in Portugal.

5. Hexaploid BBBBBB

The autohexaploid race (2n = 6x = 42) is limited to populations around Trieste in northern Italy and Hungary.

6. Hexaploid AABBBB

The autoallohexaploid race (2n = 6x = 42) is limited in its distribution to a few populations in south Cornwall around the Lizard and West Penwith peninsulas and to Guernsey and Sark in the Channel Islands.

Variation in Floral Morphology

Variation in floral morphology was studied in seven natural populations of Scilla autumnalis at three ploidy levels. The plants studied were collected as mature individuals and maintained in cultivation for up to 3 years.

i) Diploid populations (BB): Lake Korillion, Corfu (LK)

ii) Tetraploid populations (BBBB): Ivy Cove, Dartmouth (IC), Pointe de St. Gildas, Pornic (PSG), Sion-sur-l'Ocean, St. Gilles Croix de Vie (SSL), Grosnez Point, Jersey (GN).

iii) Hexaploid populations (AABBBB): Goonhilly Downs, The Lizard (GD), Les l'Aches, Sark (LL).

In addition, a synthetic population of two year old pentaploid hybrid plants (ABBBB), produced by hybridising autotetraploids and autoallohexaploids, was also examined.

Measurements of floral morphology were made on between 17 and 27 plants in each population. Seven measurements of floral structures were made on newly-opened flowers: (i) perianth part length and (ii) perianth part width; (iii) filament length; (iv) ovary length; (v) style length; (vi) pedicel length and (vii) perianth part shape expressed as the distance from

its widest part to the tip divided by the total length. All populations showed variance homogeneity (Bartlett's method) and were tested for differences in mean by analysis of variance. Populations showing significant differences in mean were compared using the Student - Newman-Keuls multiple range test in order to specify the source of such differences.

i) Perianth part length

Mean perianth part length varied from 5.1 mm (LK) to 6.34 mm in the pentaploid hybrids (Table 7.1). There were highly significant differences in mean between the populations ($F = 8.406$, $P < 0.001$; Table 7.2). The multiple range analysis shows that no populations stand out as having an exceptionally large difference in mean although the general trend is for the mean perianth length to increase with increasing chromosome number (Fig. 7.1).

ii) Perianth part width

Perianth part width varied from 1.85 mm (LK) to 3.12 mm (SSL) (Table 7.1) but there were no significant differences in mean between the populations ($F = 1.47$, $P > 0.05$; Table 7.2).

iii) Filament length

Mean filament length varied between 3.55 mm (LK) and 4.43 mm (pentaploid hybrids) (Table 7.1). There were highly significant differences in mean filament length between the populations ($F = 6.785$, $P < 0.001$; Table 7.2). Multiple range analysis *indicates two discrete groups (Fig. 7.1):* LK, PSG, SSL, IC, GN with significantly smaller filaments than LL, GD and pentaploid hybrids. Thus filament length separates populations which contain A genome chromosomes from those without.

iv) Ovary length

Mean ovary length varies between 2.07 mm (LK) and 2.54 mm (LL)(Table 7.1). There were highly significant differences between populations ($F = 5.869$,

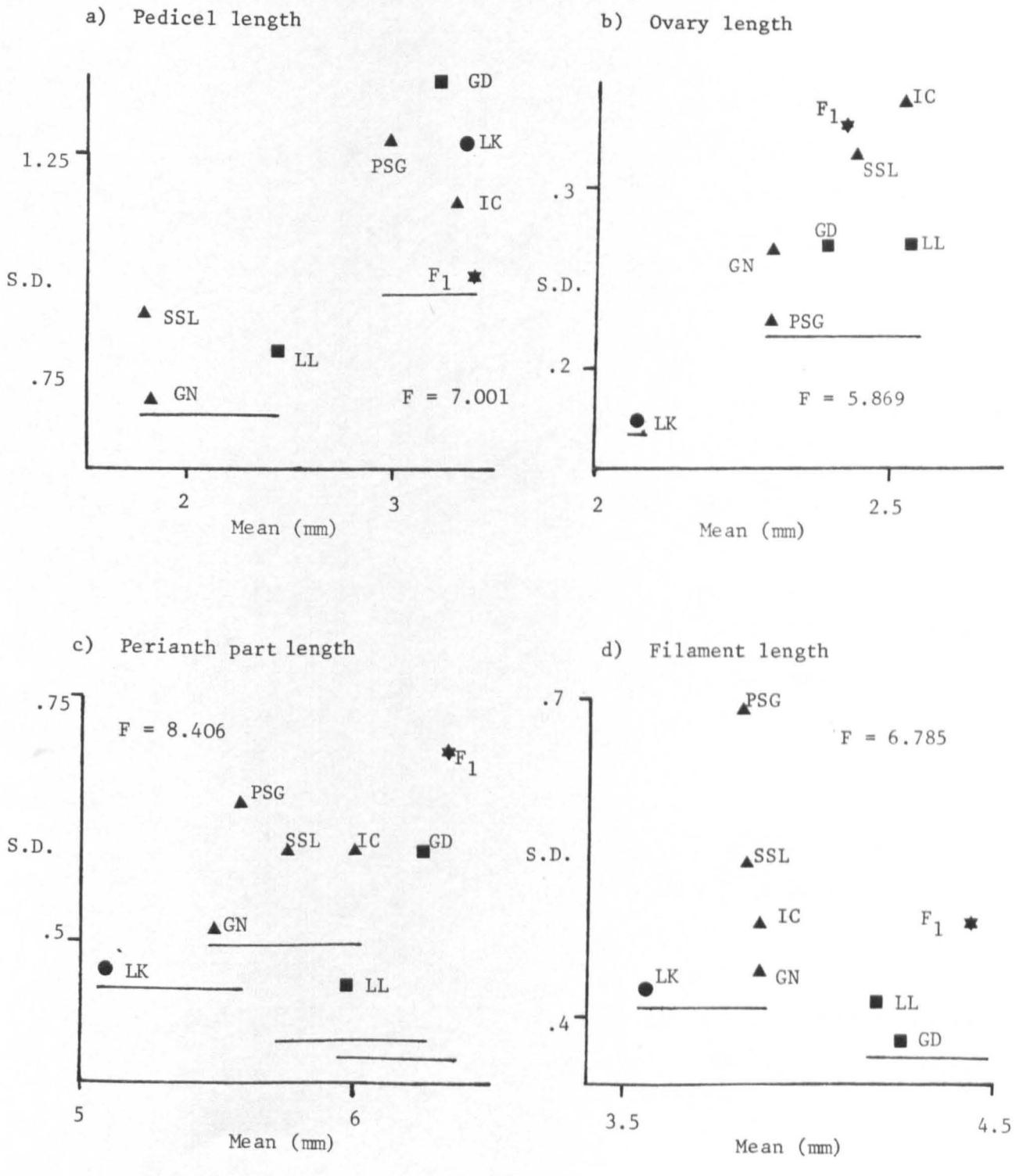
Table 7.1 Variation in seven floral characters in diploid, autotetraploid, pentaploid $F_1$ hybrid and autoallohexaploid plants of Scilla autumnalis. All characters showed homogeneity of variance (Bartlett's test)

| Character | | Population | | | | | | | | DF | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LK 2x | IC 4x | PS9 4x | GN 4x | SSL 4x | 5x | GD 6x | LL 6x | | |
| Perianth part length (mm) | Mean | 5.1 | 6.0 | 5.57 | 5.48 | 5.75 | 6.34 | 6.25 | 5.98 | | |
| | s.d. | 0.573 | 0.65 | 0.682 | 0.60 | 0.651 | 0.714 | 0.648 | 0.562 | 7 | 0.470 |
| | n | 17 | 25 | 25 | 25 | 25 | 18 | 27 | 27 | | |
| Perianth part width (mm) | Mean | 1.85 | 2.38 | 2.20 | 2.26 | 3.12 | 2.83 | 2.66 | 2.62 | | |
| | s.d. | 0.207 | 0.30 | 0.203 | 0.25 | 0.394 | 0.348 | 0.238 | 0.30 | 7 | 3.921 |
| | n | 17 | 25 | 17 | 25 | 25 | 18 | 27 | 27 | | |
| Filament length (mm) | Mean | 3.55 | 3.86 | 3.81 | 3.86 | 3.82 | 4.43 | 4.24 | 4.17 | | |
| | s.d. | 0.435 | 0.45 | 0.685 | 0.494 | 0.546 | 0.495 | 0.387 | 0.422 | 7 | 2.213 |
| | n | 17 | 25 | 17 | 25 | 24 | 18 | 27 | 27 | | |
| Ovary length (mm) | Mean | 2.07 | 2.53 | 2.30 | 2.30 | 2.44 | 2.43 | 2.39 | 2.54 | | |
| | s.d. | 0.176 | 0.349 | 0.229 | 0.27 | 0.318 | 0.336 | 0.271 | 0.269 | 7 | 2.906 |
| | n | 17 | 25 | 17 | 25 | 24 | 18 | 27 | 27 | | |
| Style length (mm) | Mean | 1.96 | 2.08 | 2.04 | 1.98 | 1.88 | 2.13 | 2.10 | 2.65 | | |
| | s.d. | 0.469 | 0.334 | 0.362 | 0.305 | 0.249 | 0.363 | 0.316 | 0.316 | 7 | 2.18 |
| | n | 17 | 25 | 27 | 25 | 24 | 18 | 27 | 27 | | |
| Pedicel length (mm) | Mean | 3.34 | 3.30 | 2.98 | 1.81 | 1.78 | 3.40 | 3.20 | 2.45 | | |
| | s.d. | 1.27 | 1.14 | 1.28 | 0.736 | 0.921 | 0.989 | 1.39 | 0.842 | 7 | 3.90 |
| | n | 17 | 25 | 17 | 25 | 24 | 18 | 26 | 27 | | |
| Perianth part shape ratio | Mean | 0.453 | 0.441 | 0.467 | 0.456 | 0.452 | 0.454 | 0.488 | 0.441 | | |
| | s.d. | 0.071 | 0.113 | 0.052 | 0.052 | 0.057 | 0.065 | 0.046 | 0.067 | 7 | 6.90 |
| | n | 16 | 25 | 17 | 25 | 25 | 17 | 27 | 27 | | |

Table 7.2 Analysis of variance of seven floral characters in diploid, autotetraploid, pentaploid $F_1$ hybrid and autoallohexaploid plants of S. autumnalis

| Floral Character | Sums of squares | | | | | | Mean squares | | F | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | (DF) | Between Populations | (DF) | Between Plants | (DF) | Between Populations | Between Plants | | |
| Perianth part length | 95.19 | (180) | 24.16 | (7) | 71.03 | (173) | 3.45 | 0.41 | 8.406 | <0.001 |
| Perianth part width | 401.42 | (180) | 23.04 | (7) | 387.37 | (173) | 3.29 | 2.24 | 1.470 | >0.05 |
| Filament length | 52.87 | (179) | 11.441 | (7) | 41.43 | (172) | 1.63 | 0.24 | 6.785 | <0.001 |
| Ovary length | 17.05 | (179) | 3.28 | (7) | 13.76 | (172) | 0.470 | 0.08 | 5.869 | <0.001 |
| Style length | 20.41 | (178) | 1.11 | (7) | 19.31 | (171) | 0.159 | 0.11 | 1.404 | >0.05 |
| Pedicel length | 332.43 | (178) | 74.09 | (7) | 258.34 | (171) | 10.59 | 1.51 | 7.005 | <0.001 |
| Perianth part shape ratio | 0.856 | (178) | 0.042 | (7) | 0.814 | (171) | 0.006 | 0.0048 | 1.259 | >0.05 |

Figure 7.1 Multiple range analysis of four floral characters in diploid (●), autotetraploid (▲), $F_1$ pentaploid hybrid (✱) and auto-allohexaploid (■) populations of Scilla autumnalis. Groups of populations not exhibiting significant differences in mean ($P > 0.05$) are plotted above a common bar line.



$P < 0.001$; Table 7.2) and a multiple range analysis separates the LK population from the remaining 7 populations (Fig. 7.1).

v) Style length

Mean style length varied from 1.88 mm (SSL) to 2.65 mm (LL) but no significant differences were found ($F = 1.404$, $P > 0.05$; Table 7.2).

vi) Pedicel length

Mean pedicel length varied between 1.78 mm (SSL) and 3.4 mm (pentaploid hybrids) (Table 7.1) with significant differences between the populations ($F = 7.005$, $P < 0.001$; Table 7.2). Although multiple range analysis splits these populations into two distinct groups there appears no consistent relationship with ploidy level (Fig. 7.1).

vii) Perianth part shape

Mean perianth part shape (ratio) was very similar in all populations only varying between 0.441 (IC, LL) and 0.488 (GD) (Table 7.1) with no significant differences ($F = 1.259$, $P > 0.05$; Table 7.2).

Variation in seed weight

The mean seed weights of 10 autotetraploid and 10 autoallohexaploid plants were almost identical with 1.91 mg and 1.96 mg respectively ($t = 0.216$, $P > 0.8$; Table 7.3).

Variation in pollen grain size

Measurements of pollen size were made on five randomly selected plants, 30 grains in each, from each of four races (BB, BBBB, ABBBB hybrids, and AABBBB).

Scilla autumnalis pollen is ellipsoidal in shape and, when squashed, the grain increases in width rather than length. Because of differential

Table 7.3 Mean seed weights of autotetraploid and autoallohexaploid plants of S. autumnalis (50 seeds from each plant)

| 4x | | 6x | |
|---|---|---|---|
| Plant | Mean weight (mg) | Plant | Mean weight (mg) |
| SG1/14 | 2.22 | LC5 | 1.36 |
| SG3/18 | 1.83 | LC9 | 2.25 |
| SG5/16 | 1.02 | LC14 | 1.62 |
| SG5/21 | 1.70 | SMP2 | 2.25 |
| IC53 | 2.08 | CT22 | 2.51 |
| MP27 | 2.05 | CT23 | 2.01 |
| ICM7 | 1.67 | GD3 | 1.67 |
| LQP33 | 1.52 | GD20 | 2.17 |
| CCF10 | 2.14 | GD23 | 1.98 |
| PP25 | 2.92 | GD30 | 1.73 |
| Mean | 1.91 | | 1.96 |
| s.d. | 0.502 | | 0.354 |

$t = 0.216 \quad (P > 0.8)$

squashing of the pollen it was not practicable to estimate volume, and length was used as an estimate of volume. This problem could perhaps be overcome by mounting in a stain containing glycerol or lactic acid, or alternatively by using a Coulter counter.

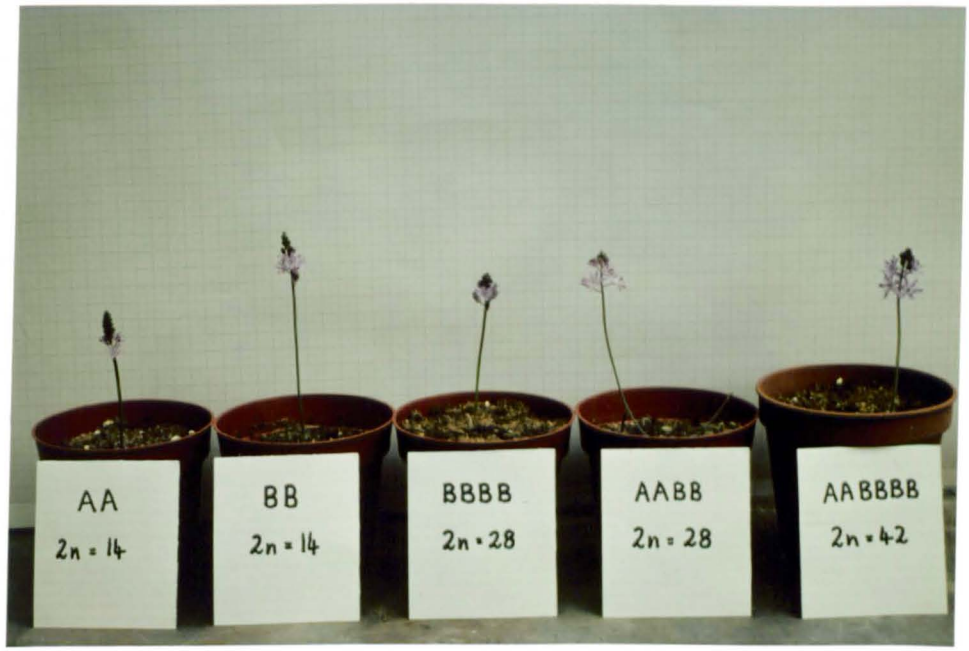
Mean pollen grain length increased from 43.1 μm in diploids to 48.8 μm in hexaploids (Table 7.4) and the differences were significant ($F = 6.334$, $P < 0.01$; Table 7.5). There were no significant differences in pollen grain length between individuals within ploidy levels. There is a significant positive regression between pollen grain length and ploidy level expressed as total mitotic chromosome length ($t = 0.6917$, $P < 0.001$; Fig. 7.3). This increase of 5.7 μm (13%) between diploids and hexaploids, however, is extremely small by comparison with the increase in total chromosome length of 243%.

Summary of morphological variation

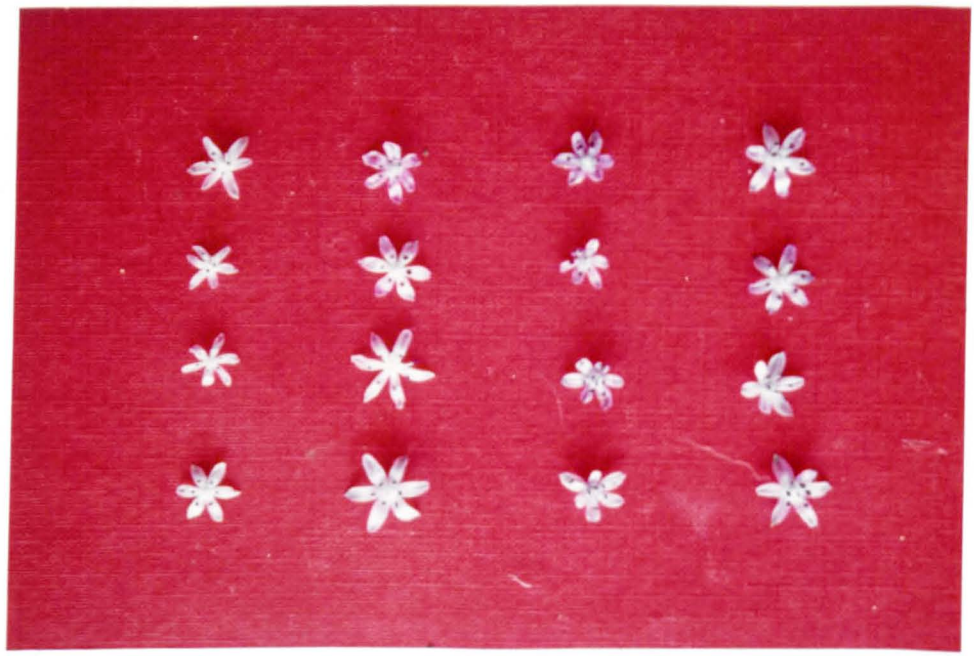
Of the four floral morphology characters which showed significant differences between populations, no single character separates the races successfully, though ovary length and filament length are each in part diagnostic. Plotting mean ovary length against mean filament length results in three groups of populations (Fig. 7.2): the diploid BB population, the tetraploid populations, and the A genome containing populations. (The inclusion of pedicel length does not aid the separation (see Plate 7.1).

Although seed weight does not differ significantly between autotetraploids and autoallohexaploids pollen grain size does increase significantly with ploidy level between the three races. In Leopoldia the diploids, autotetraploids and autohexaploids of four species were found to be morphologically indistinguishable although the levels of ploidy were to some extent

Plate 7.1 Morphology of chromosome races of Scilla autumnalis




(a) Inflorescences



BB BBBB ABBBB AABBBB

(b) Single flowers

Table 7.4 Pollen grain length in diploid, autotetraploid, pentaploid $F_1$ hybrid and autoallohexaploid plants of S. autumnalis. (Mean of 5 plants, 30 grains per plant.)

| Pollen grain length (μm) | Ploidy level | | | |
|---|---|---|---|---|
| | 2x | 4x | 5x | 6x |
| Mean | 43.1 | 44.6 | 47.0 | 48.8 |
| s.d. | 0.14 | 0.28 | 0.29 | 0.26 |

Table 7.5 Analysis of variance of pollen grain length in diploid, autotetraploid, pentaploid $F_1$ hybrid and autoallohexaploid plants of S. autumnalis

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between ploidy levels | 3 | 96.956 | 32.322 | 6.334 | <0.01 |
| Between plants | 16 | 81.644 | 5.103 | | |
| Total | 19 | 178.610 | | | |

Figure 7.2 Mean ovary length against mean filament length for diploid (○), autotetraploid (△), $F_1$ pentaploid hybrid (✡), and autoallohexaploid (□) plants of S. autumnalis. Pedicel length is shown as a vertical bar.

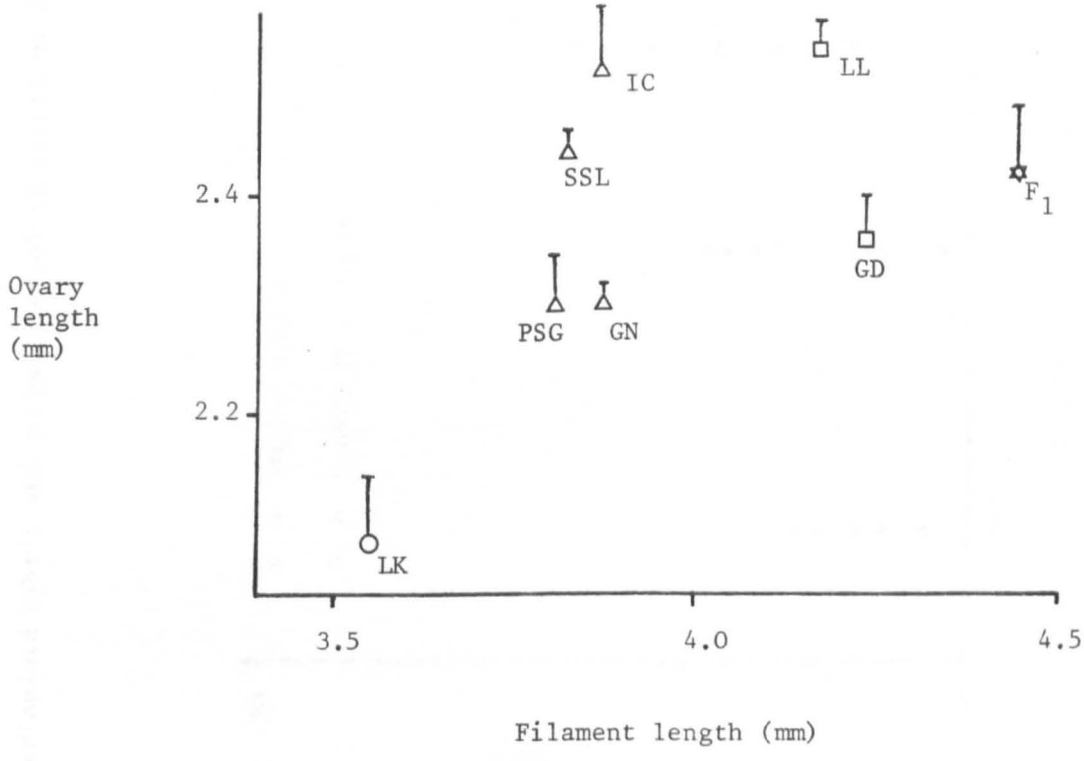
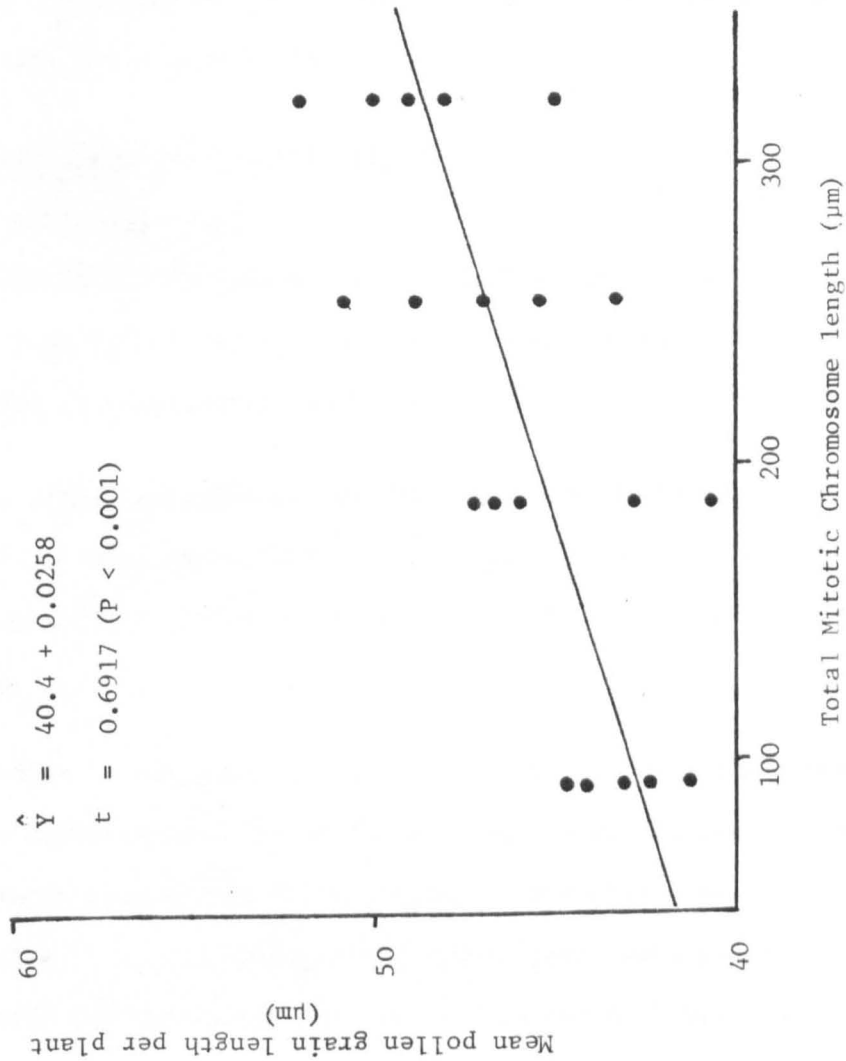


Figure 7.3 Regression of pollen grain length against mitotic chromosome length for diploid, autotetraploid, $F_1$ pentaploid hybrid and autoallohexaploid plants of S. autumnalis.



distinguishable on the basis of seed and pollen size (Bentzer, 1974). In general, natural polyploid complexes are not distinguishable on the basis of pollen size, indicating strong stabilising selection for this character.

Clearly, much of the observed variation between S. autumnalis populations will be environmental in origin and seed progeny are at present being grown up under uniform environmental conditions to establish whether the variation in these characters has a genetic basis.

Breeding behaviour under experimental conditions

a) Spontaneous self-pollination

Flower spikes of autotetraploids and autoallohexaploids were enclosed in insect-proof bags during the period in which the flowers were open to estimate the level of spontaneous selfing.

In general, autoallohexaploids are more self-compatible than autotetraploids although the degree of self-compatibility varies. 88% of hexaploids but only 54% of tetraploids set some seed under conditions of enforced selfing.

The mean number of capsules set as a proportion of the total number of flowers in an inflorescence (referred to hereafter as the proportion of capsules/flowers) ranged from 0 (19 plants) to 0.6 with a mean of 0.1 in tetraploids (Fig. 7.4). In hexaploids, fewer plants were devoid of capsules (4 plants) and the maximum set was 0.8 capsules/flowers, with a higher mean of 0.275.

The number of seeds in a capsule ranged from 1-6 in both tetraploids and hexaploids (Fig. 7.5). The mean number of seeds per capsule however was higher in hexaploids (3.4) than in tetraploids (2.8).

Figure 7.4 The mean proportion of capsules set per flower of autotetraploid and autoallohexaploid S. autumnalis plants under conditions of spontaneous and manual self-pollination

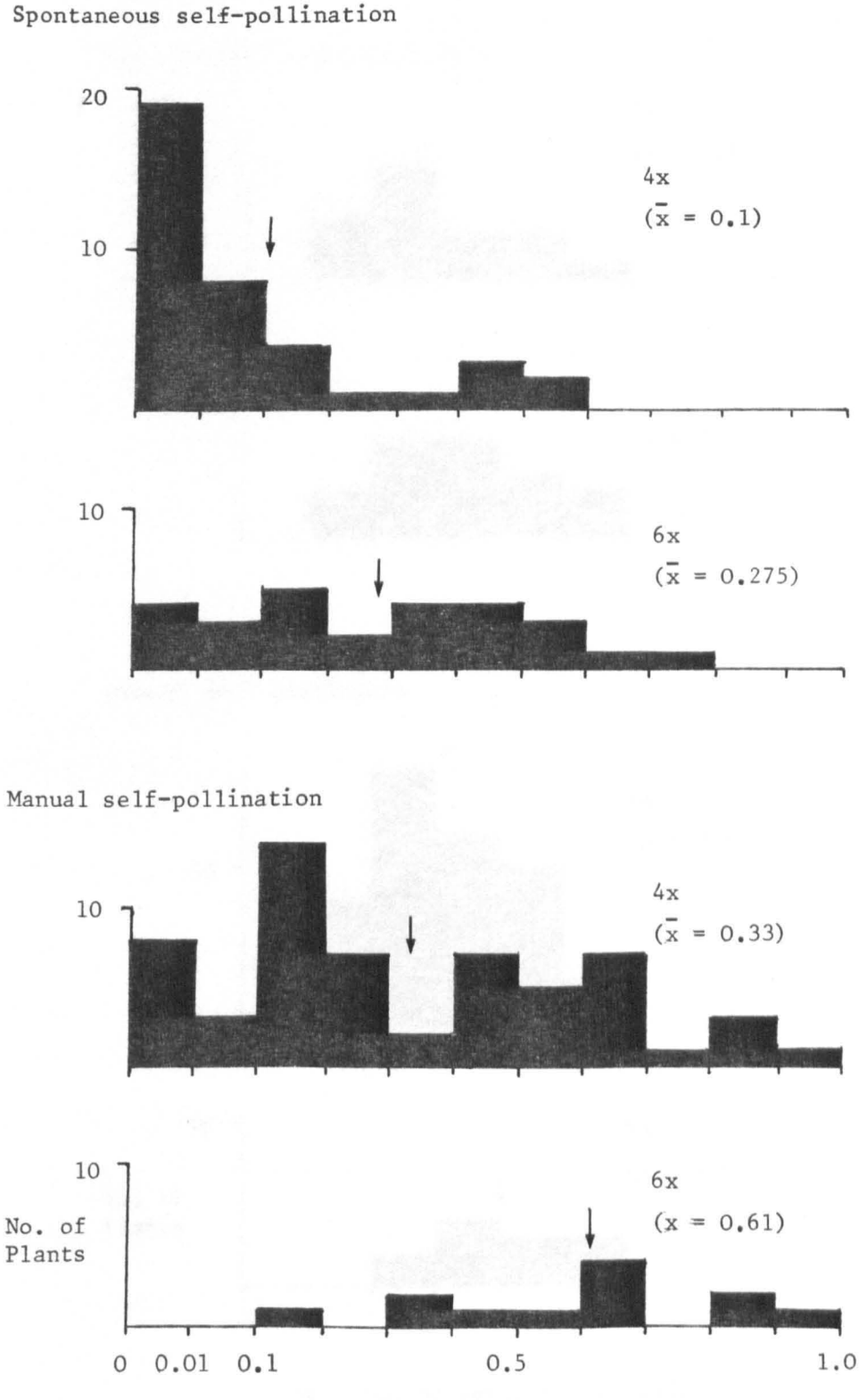
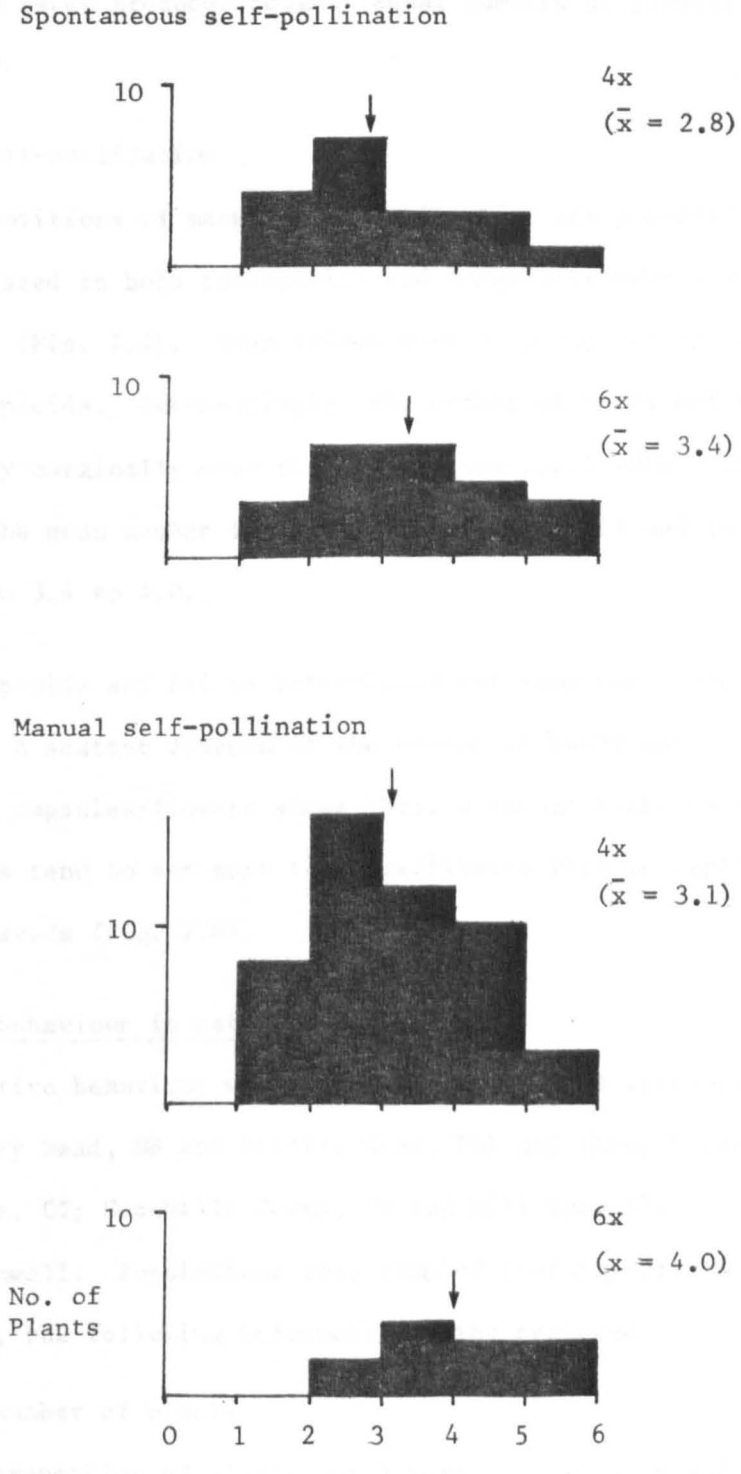


Figure 7.5 The mean number of seeds per capsule of autotetraploid and autoallohexaploid S. autumnalis plants under conditions of spontaneous and manual self-pollination



Autoallohexaploids, therefore, set seed more freely than autotetraploids when selfing is spontaneous with a higher proportion of capsules/flowers and more seeds in each capsule. The maximum number of seeds set in a single inflorescence was 47 in hexaploids and 32 in tetraploids, although both races produced roughly equal numbers of flowers in an inflorescence.

b) Manual self-pollination

Under conditions of manual self-pollination the proportion of capsules/flowers increased in both tetraploids and hexaploids over the spontaneous selfing level (Fig. 7.4). Mean values were 0.33 for tetraploids and 0.61 for hexaploids. Interestingly, the number of seeds set per capsule increased only marginally over the spontaneous level (Fig. 7.5). In tetraploids the mean number increased from 2.8 to 3.1 and in hexaploids increased from 3.4 to 4.0.

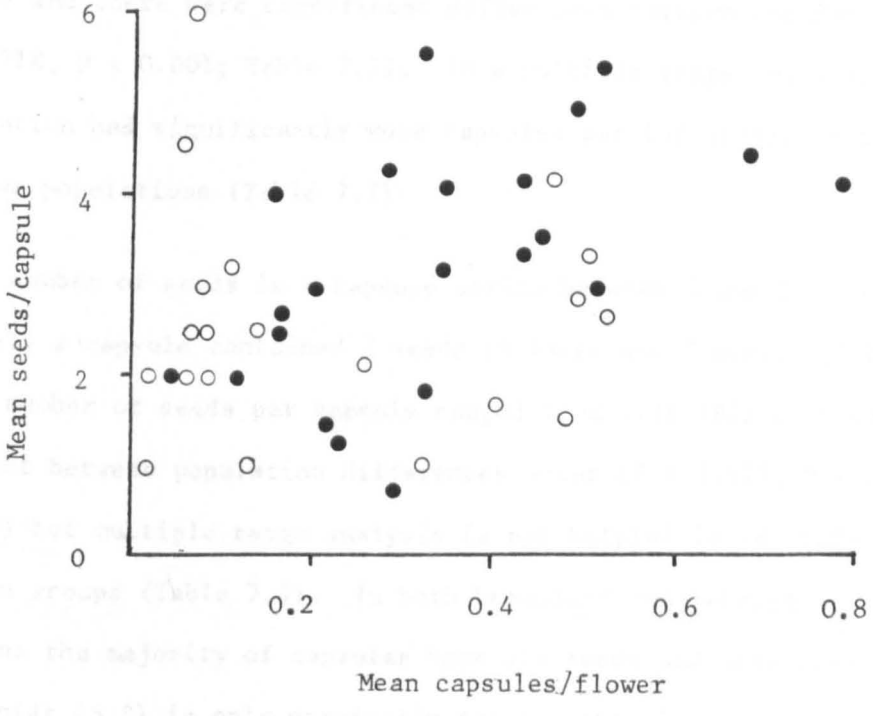
All hexaploids and 84% of tetraploids set some seed under manual self-pollination. A scatter diagram of the number of seeds per capsule against the number of capsules/flowers shows that, although there is much overlap, the hexaploids tend to set more capsules/flowers than tetraploids and these contain more seeds (Fig. 7.6).

Reproductive behaviour in natural populations

Reproductive behaviour was investigated in three tetraploid (Barras Nose, BN; Berry Head, BH and Pentire Head, PH) and three hexaploid (Caerleon Cove, CT; Goonhilly Downs, GD and Rill Top, RT) populations in Devon and Cornwall. Populations were sampled randomly with a 25 cm x 10 cm frame quadrat, the following information being recorded:-

1. the number of plants
2. the proportion of plants which were flowering or had flowered

Figure 7.6 Mean seeds per capsule against mean capsules per flower for autotetraploid (○) and autoallohexaploid (●) plants of S. autumnalis



3. the number of capsules on each inflorescence (20-50 inflorescences were sampled randomly in each population)
4. the number of seeds per capsule (capsules were sampled randomly from each inflorescence).

The proportion of flowering plants ranged between 13.6% (BN population, Table 7.6) and 44% (CT population) with the values for the hexaploid populations all being higher than those for the tetraploid.

The number of capsules per inflorescence varied between 0 and 19 (Table 7.10). The mean number per population ranged from 3.88 (BN) to 8.39 (GD) and there were significant differences between populations ($F = 10.718$, $P < 0.001$; Table 7.7). In a multiple range analysis the GD population had significantly more capsules per inflorescence than the other five populations (Table 7.7).

The number of seeds in a capsule varied between 0 and 6 although very rarely a capsule contained 7 seeds (5 large and 2 small) (Table 7.8). The mean number of seeds per capsule ranged from 4.31 (BN) to 5.63 (GD). Significant between population differences occur ($F = 3.943$, $P < 0.01$; Table 7.9) but multiple range analysis is not helpful in identifying population groups (Table 7.9). In both hexaploid and tetraploid populations the majority of capsules have six seeds and mean seed number in hexaploids (5.3) is only marginally greater than in tetraploids (4.9) (Fig. 7.7).

Interestingly, the GD population, with higher values for both numbers of capsules/inflorescence and number of seeds per capsule is an inland population and is, therefore, less exposed than the other five cliff top populations.

Table 7.6 The numbers of capsules per inflorescence in plants of three autotetraploid and three autoallohexaploid natural populations of Scilla autumnalis.

| No. of capsules/ inflorescence | Number of inflorescences | | | | | |
|---|---|---|---|---|---|---|
| | 4x | | | 6x | | |
| | BN | BH | PH | CT | GD | RT |
| 0 | - | 2 | 1 | 2 | - | - |
| 1 | 3 | 11 | 1 | 4 | - | 6 |
| 2 | 5 | 9 | 2 | 2 | 2 | 7 |
| 3 | 5 | 15 | 5 | 4 | 6 | 5 |
| 4 | 3 | 12 | 8 | 5 | 6 | 9 |
| 5 | 5 | 7 | 3 | 2 | 2 | 6 |
| 6 | 2 | 4 | 4 | 1 | 2 | 8 |
| 7 | 2 | 2 | 3 | 4 | 1 | 3 |
| 8 | - | 5 | 3 | - | 4 | 2 |
| 9 | - | 1 | - | - | 5 | 1 |
| 10 | 1 | 1 | 1 | 2 | 5 | 1 |
| 11 | - | 1 | 1 | 1 | 3 | - |
| 12 | - | 1 | - | - | 2 | - |
| 13 | - | 1 | - | 1 | 2 | 1 |
| 14 | - | - | - | - | 1 | 1 |
| 15 | - | - | - | 1 | 2 | - |
| 17 | - | 1 | - | - | - | - |
| 19 | - | - | - | - | 3 | - |
| Total inflorescences | 26 | 73 | 32 | 29 | 46 | 50 |
| Total plants | 24 | 63 | 31 | 20 | 34 | 47 |
| Mean no. of capsules/ inflorescence | 3.88 | 4.21 | 4.91 | 4.93 | 8.39 | 4.64 |
| S.D. | 2.18 | 3.17 | 2.48 | 3.87 | 4.64 | 2.88 |
| No. of plants with 1 inflorescence | 22 | 55 | 30 | 12 | 27 | 45 |
| 2 inflorescences | 2 | 6 | 1 | 7 | 2 | 1 |
| 3 inflorescences | - | 2 | - | 1 | 5 | 1 |

Bartlett's test for homogeneity of variance $\chi^2_{(5)} = 6.755$ ($P > 0.2$)

Table 7.7 Analysis of variance and multiple range analysis of the numbers of capsules per inflorescence in plants of three autotetraploid and three autotetraploid natural populations of S. autumnalis

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between populations | 5 | 606.105 | 121.221 | 10.718 | <0.001 |
| Between inflorescences | 250 | 2827.629 | 11.311 | | |
| Total | 255 | 3433.734 | | | |

Multiple range analysis

| BN | BH | RT | PH | CT | GD |
|---|---|---|---|---|---|

Table 7.8 The numbers of seeds per capsule in plants of three autotetraploid and three autoallohexaploid natural populations of S. autumnalis (1 capsule chosen at random from each plant)

| No. of seeds/ capsule | Number of capsules | | | | | |
|---|---|---|---|---|---|---|
| | | 4x | | | 6x | |
| | BN | BH | PH | CT | GD | RT |
| 0 | 1 | - | - | 1 | - | - |
| 1 | 1 | - | - | 1 | - | - |
| 2 | 3 | 4 | 1 | - | - | - |
| 3 | 2 | 3 | 3 | 1 | 1 | 2 |
| 4 | 5 | 9 | 3 | 2 | 2 | 8 |
| 5 | 5 | 10 | 9 | 4 | 7 | 10 |
| 6 | 9 | 23 | 14 | 11 | 24 | 27 |
| 7 | - | - | 1 | - | 1 | - |
| Mean seeds/ capsule | 4.31 | 4.92 | 5.13 | 4.90 | 5.63 | 5.32 |
| S.D. | 1.76 | 1.29 | 1.18 | 1.74 | 0.77 | 0.91 |
| Total capsules (plants) | 26 | 49 | 31 | 20 | 35 | 47 |

Bartlett's test for homogeneity of variance $\chi^2_{(5)} = 8.12$ ( $P > 0.1$)

Table 7.9 Analysis of variance and multiple range analysis of the numbers of seeds per capsule in plants of three autotetraploid and three autoallohexaploid natural populations of S. autumnalis

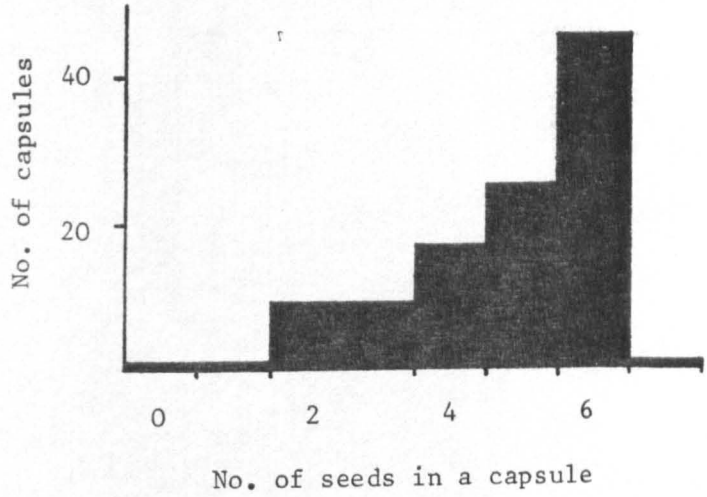
| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between populations | 5 | 30.731 | 6.146 | 3.943 | <0.01 |
| Between plants | 202 | 314.880 | 1.551 | | |
| Total | 207 | 345.611 | | | |

Multiple range analysis

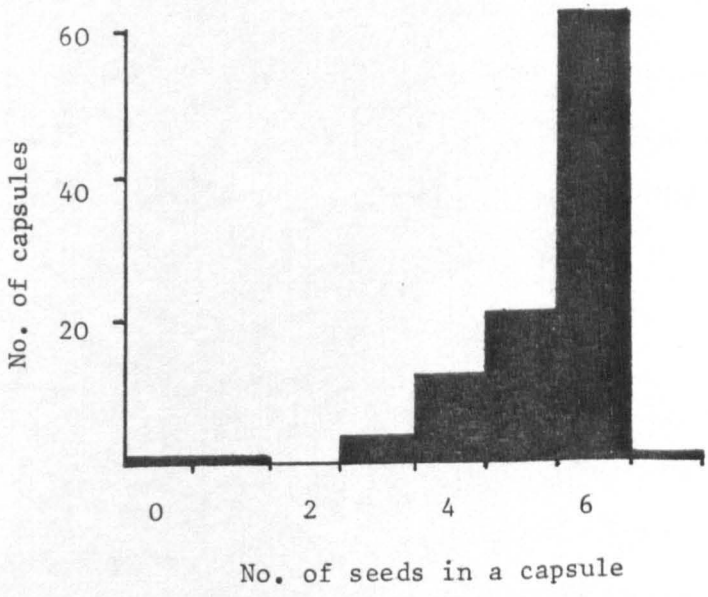
| BN | CT | BH | PH | RT | GD |
|---|---|---|---|---|---|

Figure 7.7 The frequency of capsules containing different numbers of seeds from autotetraploid and autoallohexaploid plants in natural populations of S. autumnalis

4x




6x



Hexaploid plants produce more seeds than tetraploids, the highest mean population number being GD (66.4) and the lowest BN (18.1). The combination of this high seed production with higher proportions of flowering plants gives a huge seed rain in hexaploid populations. The hexaploid populations, however, contain only about one tenth the number of plants of tetraploid populations (15,000-51,000 vs. 210,000-655,000; Table 7.10).

The estimated seed production of a population per annum is enormous, varying from 275,000 (GD 15,000 individuals) to 2,150,000 (BH, 655,000 individuals).

All observations of reproductive behaviour have been made on plants which had finished flowering and carried ripe seeds. It would be instructive to investigate these populations earlier in the year to establish the actual numbers of inflorescences and flowers produced.

Though it is clear from selfing experiments that most S. autumnalis plants are to some extent self-compatible the frequency and diversity of insect visitors suggest that the species is predominantly outcrossed in nature. Experimental verification of this could be carried out using chromosome variants as markers of gene flow.

Pollen stainability

Pollen stainability was studied in plants of four ploidy levels; diploids, autotetraploids, pentaploid hybrids and autoallohexaploids (Table 7.11). Mean pollen stainability was lowest in the diploids with 65% and highest in the hexaploids with 78%. These differences, however, were not significant ($F = 2.3035$, $P > 0.05$, Table 7.12) due to the huge variation between plants. It remains to be established whether pollen stainability can be directly equated with pollen fertility. It is clear, however, that

Table 7.10 Population structure and reproductive behaviour in three autotetraploid and three auto-allohexaploid natural populations of S. autumnalis

| | Population | Estimated density (plants/$m^2$) | Area ($m^2$) | Estimated population size | Proportion flowering (%) | Estimated no. of flowering plants | Mean seeds/ plant | Total seed production per year |
|---|---|---|---|---|---|---|---|---|
| | BN | 10-630 | 2,400 | 210,000 | 17.3 | 3,600 | 18.1 | 650,000 |
| 4x | BH | 155-161 | 3,500 | 655,000 | 13.6 | 89,000 | 24.0 | 2,136,000 |
| | PH | 200-996 | 1,150 | 373,000 | 17.6 | 66,000 | 26.0 | 1,720,000 |
| | CT | 116 | 300 | 33,000 | 44.0 | 14,500 | 35.3 | 510,000 |
| 6x | GD | 62-179 | 150 | 15,000 | 27.6 | 4,100 | 66.4 | 275,000 |
| | RT | 116 | 300 | 51,000 | 21.4 | 11,000 | 26.3 | 290,000 |

Table 7.11 Pollen stainability in diploid, autotetraploid, pentaploid $F_1$ hybrid and autoallohexaploid plants of Scilla autumnalis

| Ploidy level | Pollen stainability | | No. of plants |
|---|---|---|---|
| | Mean % | s.d. | |
| 2x | 64.98 | 19.62 | 15 |
| 4x | 72.16 | 15.28 | 64 |
| 5x | 70.54 | 14.47 | 18 |
| 6x | 77.62 | 10.14 | 24 |

Table 7.12 Analysis of variance of pollen stainability in diploid autotetraploid $F_1$ pentaploid hybrid and autoallohexaploid plants of S. autumnalis (after angular transformation)

| Source | D.F. | S.O.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Between ploidy levels | 3 | 1,544.582 | 514.861 | 2.304 | >0.05 |
| Between plants | 117 | 26,150.743 | 223.511 | | |
| Total | 120 | 27,695.325 | | | |

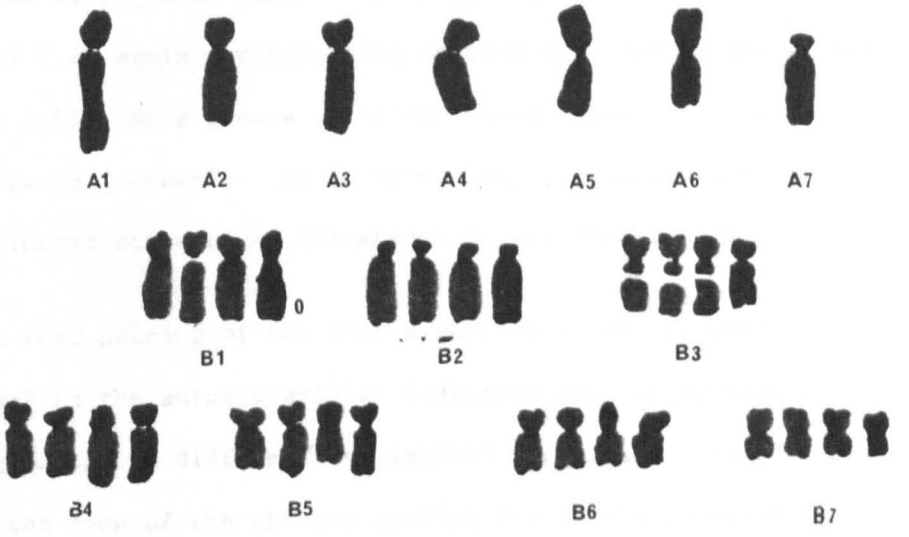
unstained grains are devoid of cytoplasm.

A common effect of induced autopolyploidy is the reduction of pollen and seed fertility in autopolyploids as compared with their diploid ancestors. For example, in autotetraploid lettuce (Einset, 1944) the quantity of seed set was reduced to 5-15% while autotetraploids of Gossypium herbaceum are completely sterile (Beasley, 1940). However, as in the case of Scilla, some autotetraploids are highly fertile, e.g. maize (Randolph, 1941) and Ehrharta erecta (Stebbins, 1949). The formation of multivalents in autopolyploids in itself does not cause a reduction of fertility (Stebbins, 1950) and physiological reasons have been put forward (e.g. Randolph, 1941). In Scilla the frequency of quadrivalent formation in autotetraploids and autoallohexaploids was low. The apparent increased fertility of polyploid Scilla might be due to the buffering of chromosomal numerical and structural variants produced during meiosis. Only cytologically 'normal' plants, as defined by the mitotic complement, were used for estimates of pollen stainability. In both Lolium perenne and Festuca pratensis (Simonsen, 1973, 1975) pollen stainability is higher in autotetraploids than in diploids, the difference being attributed to chromosomal buffering in the tetraploids since chromosomal aberrations were prevalent in both diploids and tetraploids.

$F_1$ hybrids

Hybridisation between autotetraploids and autoallohexaploids is readily carried out and results in pentaploids with 2n = 35 and genomic constitution ABBBB (Fig. 7.8). These hybrids are as viable as the parental races and have been grown to flowering in two years. Several $F_1$ hybrids have been studied at meiosis and one, BHF 3 x 9 has been analysed in detail.

Figure 7.8 The karyotype of the ABBBB $F_1$ hybrid



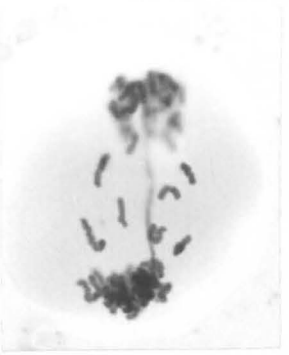
Metaphase-I behaviour of the B genome chromosomes in this $F_1$ hybrid was very similar to that found in autotetraploids. Chiasma number ranged from 26-44 chiasmata per PMC with a mean of 34.2 (Table 3.5) which is slightly, but non-significantly, higher than that in the two autotetraploids described above (p. 50 ). The distribution of chiasmata between the four identifiable choromosome groups showed the same pattern as in the diploids and tetraploids (Table 3,4; Fig. 3.9).

The number of B genome quadrivalents per PMC varied between 0 and 5 with a mean of 1.8, again corresponding exactly with values for autotetraploids (Table 3.5). No B genome univalents were observed in the 20 PMCs scored for chiasma frequency. As in tetraploids, quadrivalents were not equally distributed between the chromosome groups (Table 3.8).

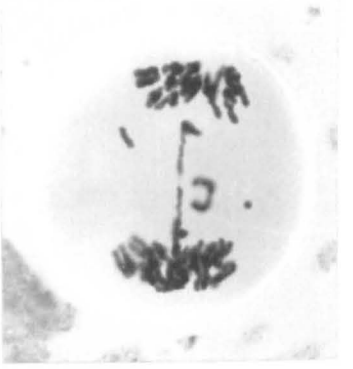
The fact that pairing of the four B genomes in the $F_1$ hybrid is so similar to that in the autotetraploids indicates that these genomes, although originating in different cytological races, are very similar. There is no indication even of the chiasma decline frequently observed in $F_1$ hybrids even with perfect pairing. Since little or no B genome chromosomal rearrangement has taken place in the evolution of the autoallohexaploid race it is likely that these races diverged in the recent past.

The A genome chromosomes in $F_1$ hybrids are always found as univalents at metaphase-I (Plate 3.1f). These univalents are readily identified as A genome by their large size. At anaphase-I, 75% of PMCs contained at least one laggard chromosome (Plate 7.2a,b) and the maximum number in a single PMC was 7 (Table 7.13). The most frequenc PMC class contained two laggard chromosomes (30%). At the dyad stage, 28% of half-dyads contained micronuclei, mostly a single micronucleus (24%), but up to four were recorded (Table 7.14).

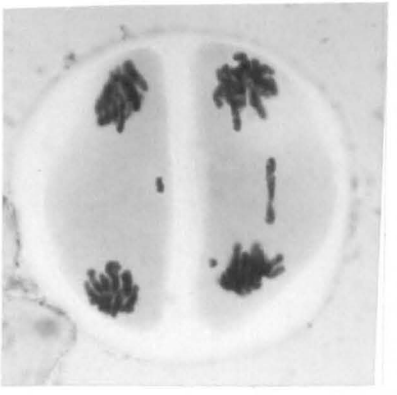
Plate 7.2 Meiosis in an $F_1$ hybrid ABBBB



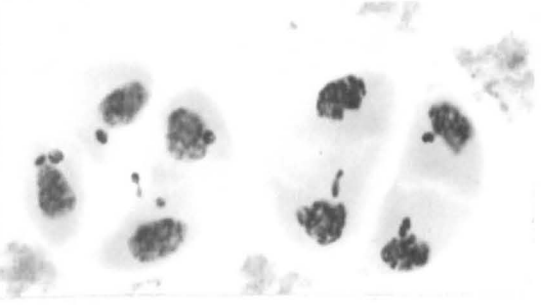
(a) A-I bridge and fragment with divided laggards



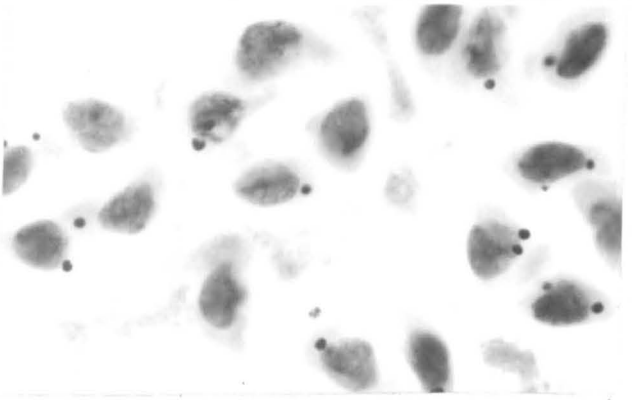
(b) A-I bridge and fragment with divided laggards



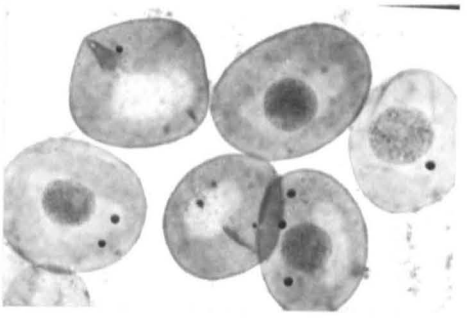
(c) T-II with fragments



(d) T-II with micronuclei



(e) Tetrads with micronuclei



(f) Young microspores with micronuclei

Table 7.13 The frequency of PMCs containing different numbers of laggard chromosomes at A-I and A-II of meiosis in an $F_1$ pentaploid hybrid plant BHF 3 x 9

| No. of laggards | No. of PMCs | |
|---|---|---|
| | A-I | A-II |
| 0 | 18 | 13 |
| 1 | 15 | 9 |
| 2 | 26 | 10 |
| 3 | 7 | 10 |
| 4 | 4 | 2 |
| 5 | - | 3 |
| 6 | 1 | 2 |
| 7 | 1 | 1 |
| Total PMCs | 72 | 50 |

Table 7.14 The numbers of cells containing different numbers of micronuclei at three stages of pollen development in the $F_1$ pentaploid hybrid plant BHF 3 x 9. Expected values calculated on the basis of a Poisson distribution

| | Developmental stage | No. of cells | | | | Mean | s.d | Total cells | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of micronuclei per cell | | | | | | | | |
| | | 0 | 1 | 2 | 3-5 | | | | | |
| Half-dyad | Obs. | 96 (72%) | 32 (24%) | 4 (3%) | 1 (1%) | 0.33 | 0.38 | 133 | 0.145 | >0.7 |
| | | | | | 5 | | | | | |
| | Exp. | 95.5 | 31.6 | 5.2 | 0.7 | | | | | |
| | | | | | 59 | | | | | |
| Microspores in tetrads | Obs. | 159 (26%) | 312 (53%) | 99 (17%) | 23 (4%) | 0.99 | 0.64 | 593 | 70.246 | <0.001 |
| | Exp. | 221.4 | 218.1 | 107.4 | 46.1 | | | | | |
| Micro-spores | Obs. | 118 (56%) | 65 (31%) | 24 (11%) | 4 (2%) | 0.59 | 0.59 | 211 | 0.960 | >0.5 |
| | Exp. | 116.7 | 69.1 | 20.5 | 4.7 | | | | | |

Pollen stainability = 87%

At anaphase-II, 74% of the PMCs contain laggards (Table 7.13). Most commonly 2 or 3 are present but up to 7 have been observed. Bridges with and without fragments were observed both at A-I and A-II (Plate 7.2). At the tetrad stage, 74% of young microspores contain micronuclei, usually one (53%) but up to five (Table 7.14; Plate 7.2e). Interestingly, in the microspores only 44% contained micronuclei (Table 7.14; Plate 7.1 ), a reduction of 30% over the tetrad stage. This would suggest either death of cells containing micronuclei or enzymic destruction of micronuclei between tetrad and microspore stages.

The distribution of cells containing micronuclei is expected to conform to a Poisson. This is the case at the dyad and microspore stages, but not at the tetrad stage ($\chi^2 = 70.246$, $P < 0.001$; Table 7.14) where an excess of cells containing one micronucleus is found. There appears no simple explanation of this phenomenon.

Despite the aberrant meiotic behaviour mean pollen stainability of $F_1$ hybrids is 71% (Table 7.11) which is not significantly different from the three cytological races ($F = 2.304$, $P > 0.05$; Table 7.12). In addition, $F_1$ hybrids set seed very freely on open pollination and frequently capsules contain 6 viable seeds.

## Discussion

### I The evolution of the complex

The primary divergence in the Scilla autumnalis complex between the A and B genomes is presumably very ancient. The present day structure of the polyploid complex, however, is probably of fairly recent origin and perhaps dates only from the later stages of the last glaciation. A scheme for the

production of the complex may then be visualised (Figs. 7.9 and 7.10).

Prior to the hybridisation and chromosome doublings which resulted in the polyploid complex, the BB diploid may have had an eastern Mediterranean distribution while the AA diploid was more westerly, perhaps inhabiting north west Africa. Both races then will have moved northwestwards in the climatic ameliorations following glacial periods and at some point or points they met, the product of subsequent hybridisation and doubling being the allotetraploid.

The production of the autotetraploid requires only a doubling of the diploid BB complement, the most likely origin being by somatic restitution. The autotetraploid has been able to exploit the more continental and Atlantic regions to the north of the diploid which at the present day is limited to the southern Mediterranean area.

The autohexaploid with its distribution centred around Trieste and the northern Balkans presumably evolved from the autotetraploid. Fusion of an unreduced tetraploid gamete with a standard diploid gamete results directly in a hexaploid zygote. This takes place relatively frequently in autotetraploid populations as demonstrated by autohexaploid and backcross derivatives. It may well be that there are further enclaves of the autohexaploid race in other parts of the distribution, perhaps in the more mountainous regions of the Balkan peninsula.

It is more difficult to theorise about the origin of the AA diploid since only a single population of this race is known. However, in view of the similar superficial morphology of the A and B genome chromosomes the diploid may be more widespread, and has not been recognised as distinct from the BB diploid.

Figure 7.9 Suggested scheme for the evolution of the chromosome races of S. autumnalis (* denotes presence of Inv 3-1)

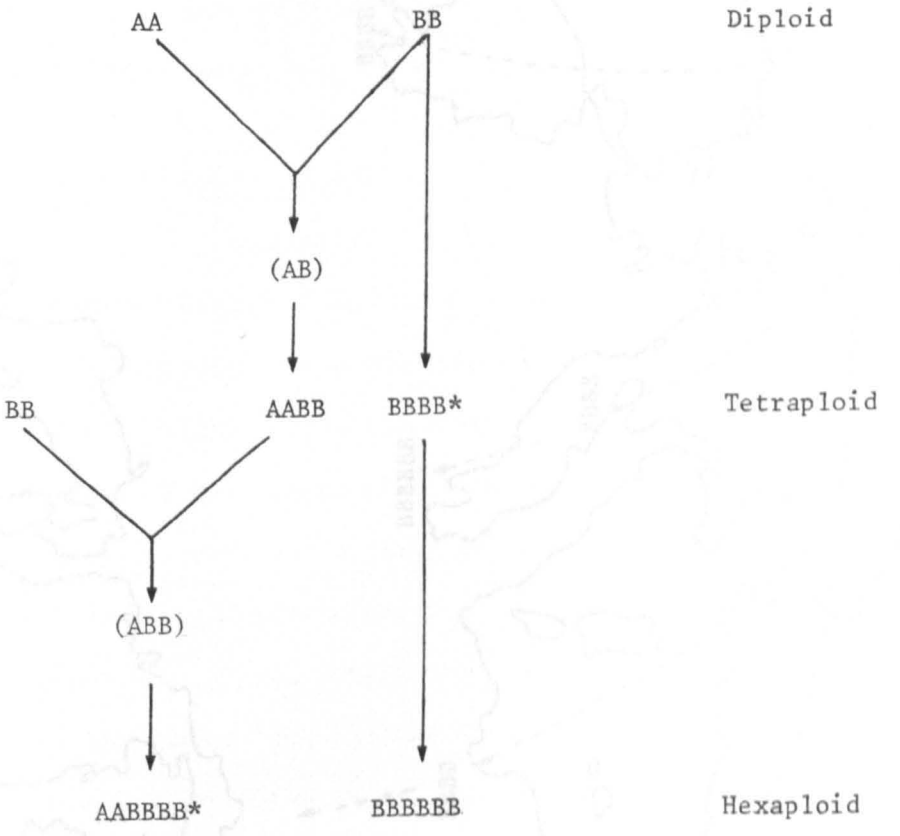
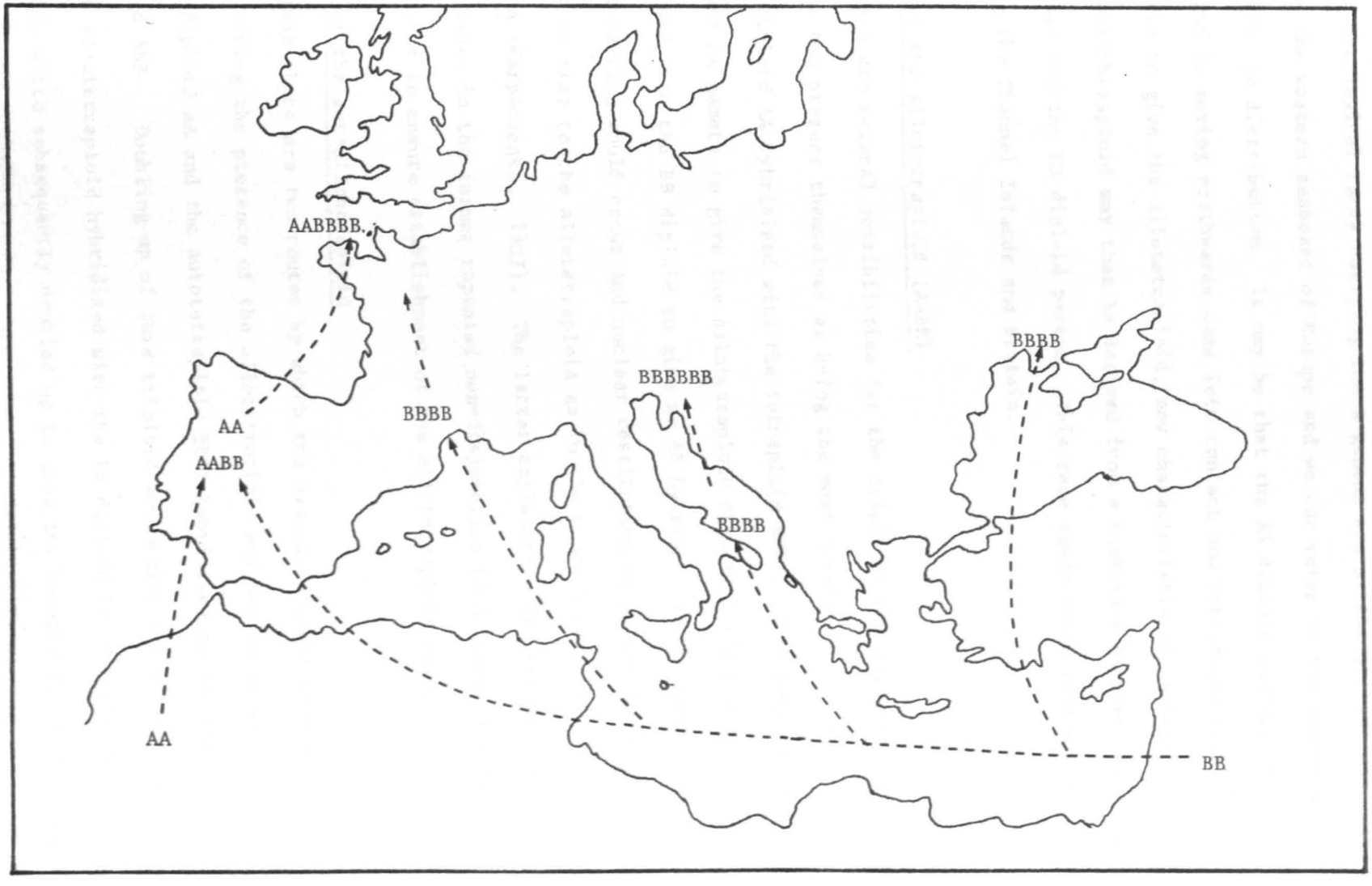


Figure 7.10 Hypothetical scheme of movement of S. autumnalis races during the recent evolution of the complex



All cytological races carrying the A genome are at the present day found on the western seaboard of Europe and we can refer to the genome as Lusitanian in distribution. It may be that the AA diploid evolved in Africa and in moving northwards came into contact and hybridised with the BB diploid to give the allotetraploid, now characteristic of Portugal. The autoallohexaploid may then be derived from a backcross between the allotetraploid and the BB diploid parent. This race again moved northwards reaching the Channel Islands and Britain.

Origin of the allotetraploid (AABB)

There are several possibilities for the origin of the allotetraploid although two present themselves as being the most likely (Fig. 7.11): i) the diploid AA hybridised with the tetraploid BBBB. This requires an unreduced AA gamete to give the allotetraploid directly; ii) the AA diploid hybridised with the BB diploid to give an AB hybrid. In the hybrid no meiotic pairing would occur and nuclear restitution in pollen and eggs would give rise to the allotetraploid as in the production of Raphano-Brassica (Karpechenko, 1927). The latter explanation is perhaps the most likely since in the former repeated non-disjunction in a standard diploid is required to ensure establishment of the allotetraploid race.

Origin of the autoallohexaploid

Again there are two routes by which the hexaploid might have evolved, one requiring the presence of the allotetraploid and the other not (Fig. 7.12): i) the diploid AA and the autotetraploid BBBB hybridised to produce a triploid ABB. Doubling up of this triploid would give the autoallohexaploid; ii) the allotetraploid hybridised with the BB diploid to give the ABB triploid, which subsequently doubled up to give the hexaploid. In the light

Figure 7.11 Possible schemes for the production of the allotetraploid race of S. autumnalis

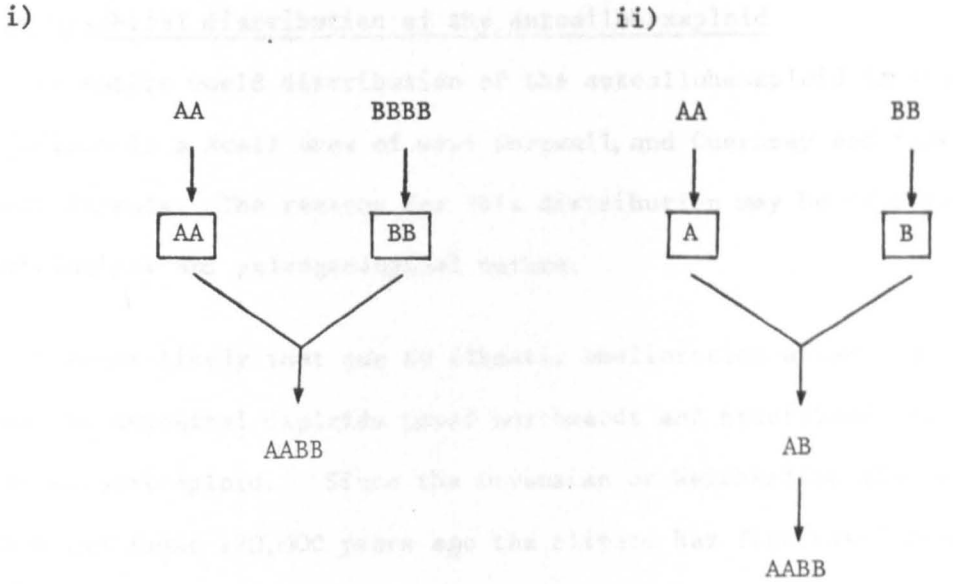
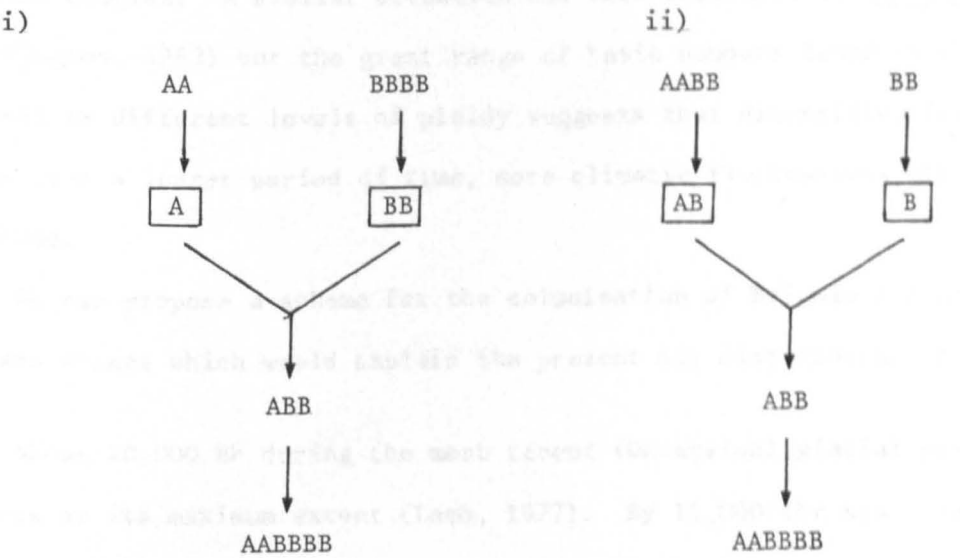



Figure 7.12 Possible schemes for the production of the autoallohexaploid race of S. autumnalis



of the present day distribution of the allotetraploid and the autoallo-hexaploid, evolution via the allotetraploid is favoured.

The geographical distribution of the autoallohexaploid

The entire world distribution of the autoallohexaploid is limited to populations in a small area of west Cornwall, and Guernsey and Sark in the Channel Islands. The reasons for this distribution may be of a paleo-climatological and paleogeological nature.

It seems likely that due to climatic amelioration after a glacial period the ancestral diploids moved northwards and hybridised resulting in the allotetraploid. Since the Devensian or Weichselian glacial period which began about 120,000 years ago the climate has fluctuated considerably as shown by assemblages of fossil Coleoptera (Coope, 1977). The most important warm periods have been the Chelford, Upton Warren and Windermere interstadials together with the present post-glacial period (Fig. 7.14) and July mean temperatures have fluctuated between about 8°C and 19°C. During these warmer periods it is likely that all the races migrated northwards but movement was reversed when the climate deteriorated again. Movement has been associated with novel hybridisation and has led to the evolution of this complex. A similar situation has been described in Cyclamen (Darlington, 1963) but the great range of basic numbers found in this genus as well as different levels of ploidy suggests that diversification has taken place over a longer period of time, more climatic fluctuations having been involved.

We can propose a scheme for the colonisation of Britain and north-western France which would explain the present day distribution of the species.

About 20,000 BP during the most recent (Devensian) glacial period the ice was at its maximum extent (Lamb, 1977). By 15,000 the sea level was at its lowest (-120m) (Fig. 7.15) and the English Channel was not in evidence

Figure 7.13 The origin and meiotic behaviour of $F_1$ pentaploid hybrids in S. autumnalis and the breeding consequences of backcrossing and inter-se mating.

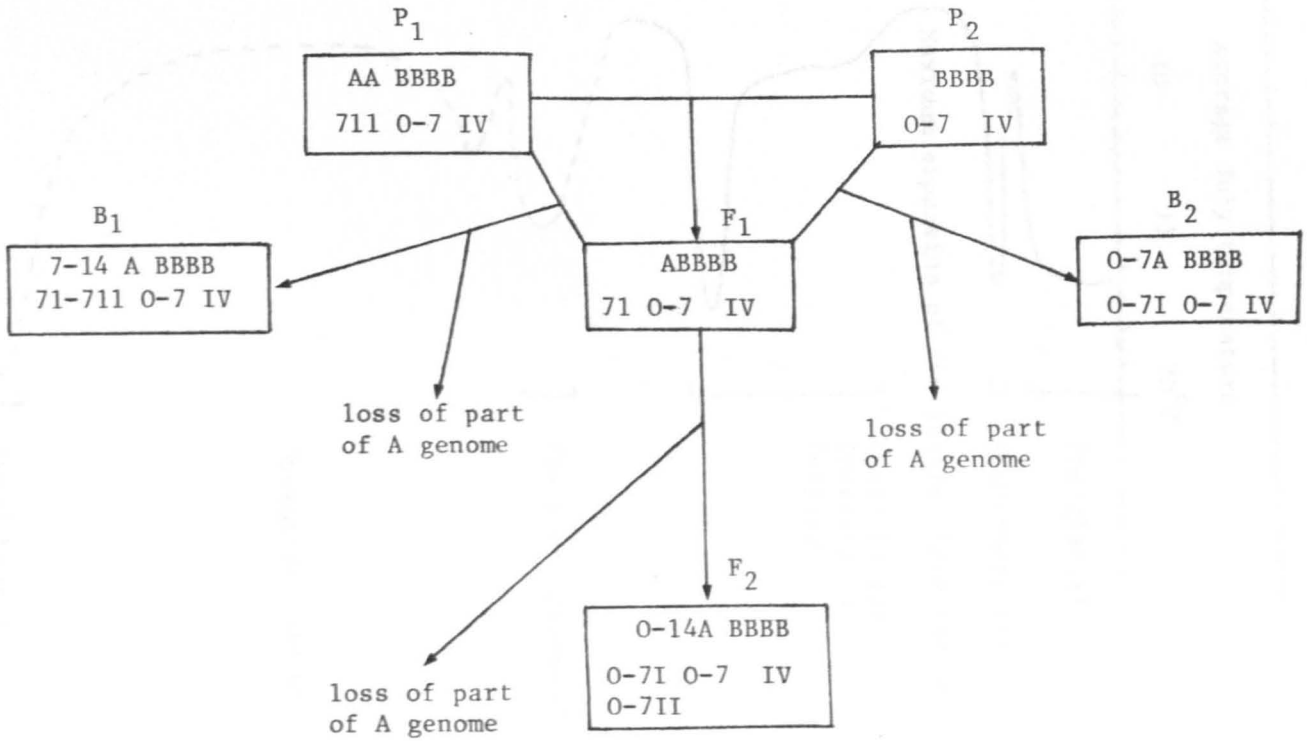


Figure 7.14 Variations in the summer temperatures in lowland central Britain since the last interglacial, based on evidence of the fossil Coleoptera (after Coope, 1977).

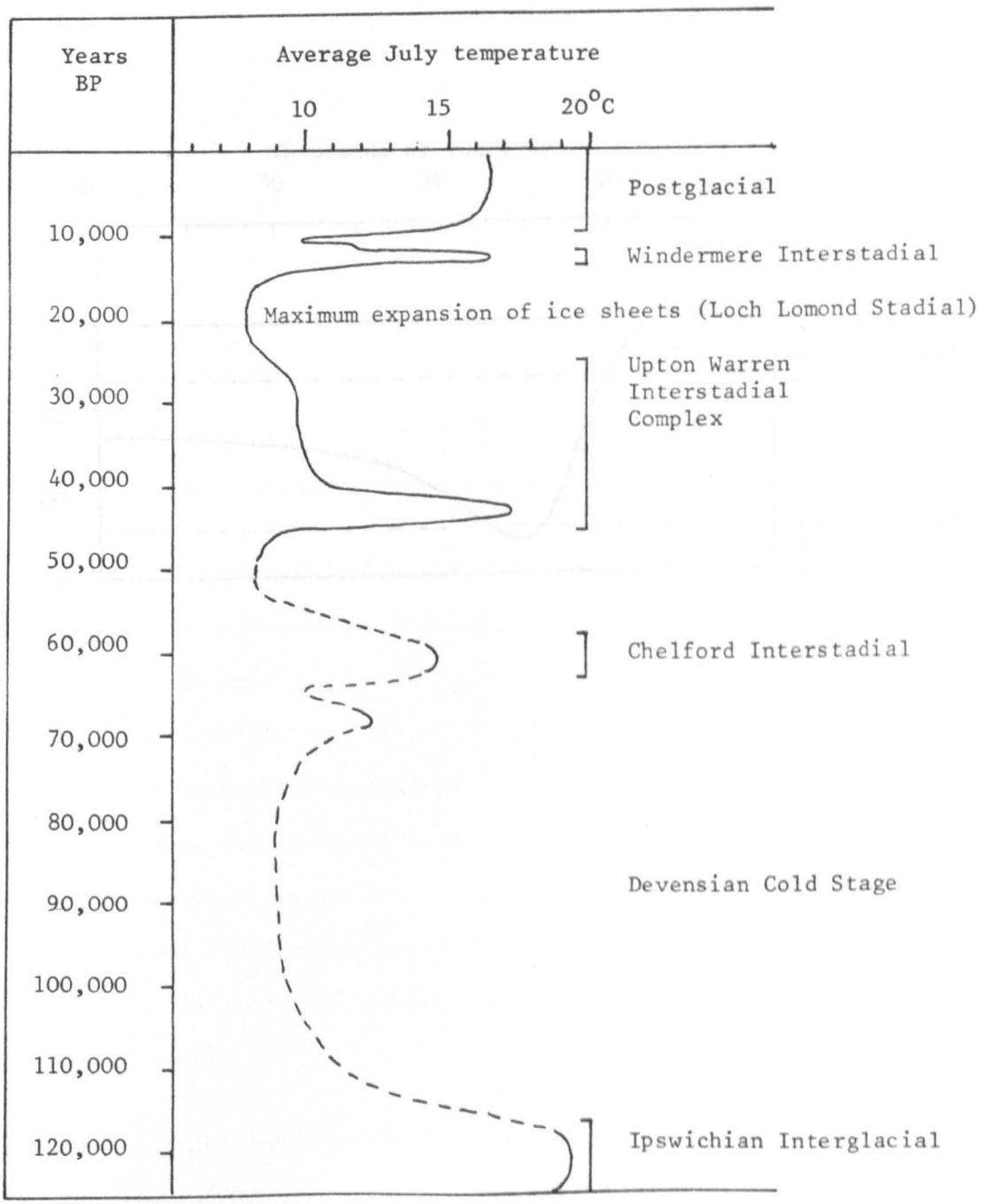
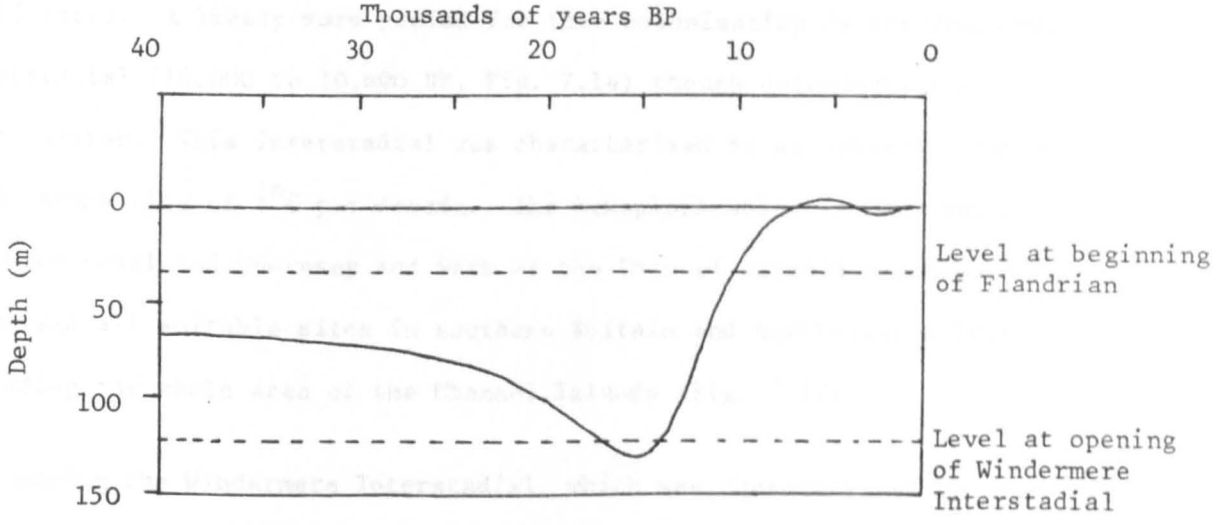


Figure 7.15 Curve for the movement of sea-level from the middle Devensian to the present (after Mitchell, 1977)



except for the Hurd Deep and areas at the extreme western (Atlantic) end of the Channel (Fig. 7.17a). Both Britain and the Channel Islands were thus part of the continental land mass at this time.

It is proposed that the initial colonising race of S. autumnalis in northern France and southern England was the autoallohexaploid AABBBB which moved northwards from western Europe (Spain and Portugal) as the climate ameliorated. A likely warm period for this colonisation is the Windermere Interstadial (14,000 to 10,600 BP, Fig. 7.14) though colonisation may have been earlier. This interstadial was characterised by an extremely rapid temperature rise of $1^{o}C$ per decade. The hexaploid which is now confined to South Cornwall and Guernsey and Sark of the Channel Islands may then have colonised all suitable sites in southern Britain and northwestern France including the whole area of the Channel Islands (Fig. 7.17).

During the Windermere Interstadial, which was characterised by mean July temperatures of up to $17^{o}C$ (Coope, 1977; Fig. 7.14) the sea level rose relatively rapidly (Mitchell, 1977; Fig. 7.15). By about 11,000 B.P. the movement of hexaploids to the western half of southern England would have been prevented by a sea mass which extended from between Normandy and the Isle of Wight to the Atlantic at the western end of the English Channel (Fig. 7.17b).(In view of the proposed northwards spread of the hexaploid it is unlikely that movement to eastern Britain across land to the east of Normandy would have occurred). At this time, a channel between Guernsey/Sark and Jersey was beginning to open (Figs. 7.16, 7.17).

The present day sea depth between Sark and Jersey is 55 m (Admiralty Chart No. 2669). At about 10,000 B.P. at the beginning of the Flandrian Interglacial in the deglaciation following the Loch Lomond stadial (Fig. 7.14) this depth of 55 m was exceeded as shown by sea level curves (Fig. 7.15; Mitchell 1977).

Figure 7.16 Map of the Channel Islands and the Armorican Peninsula of France showing the dates at which sea channels opened.

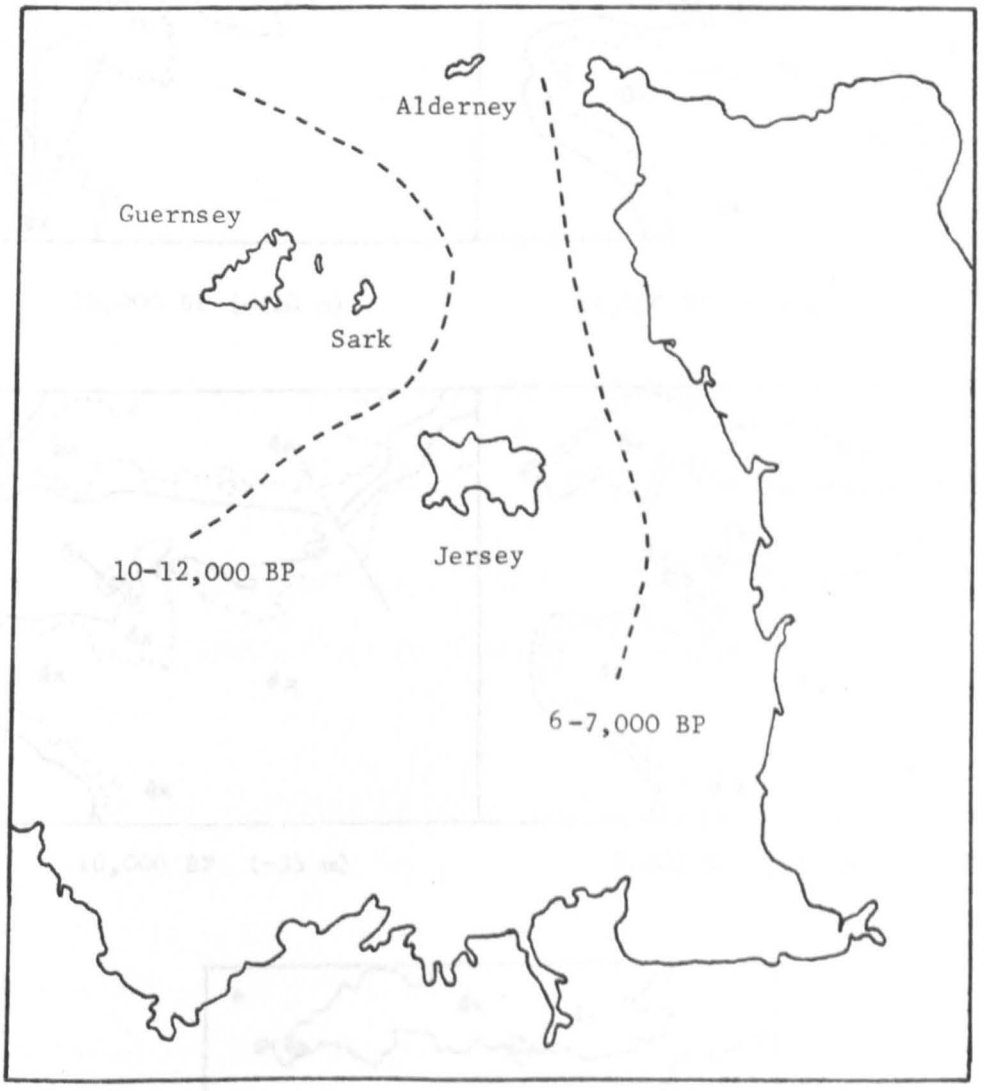
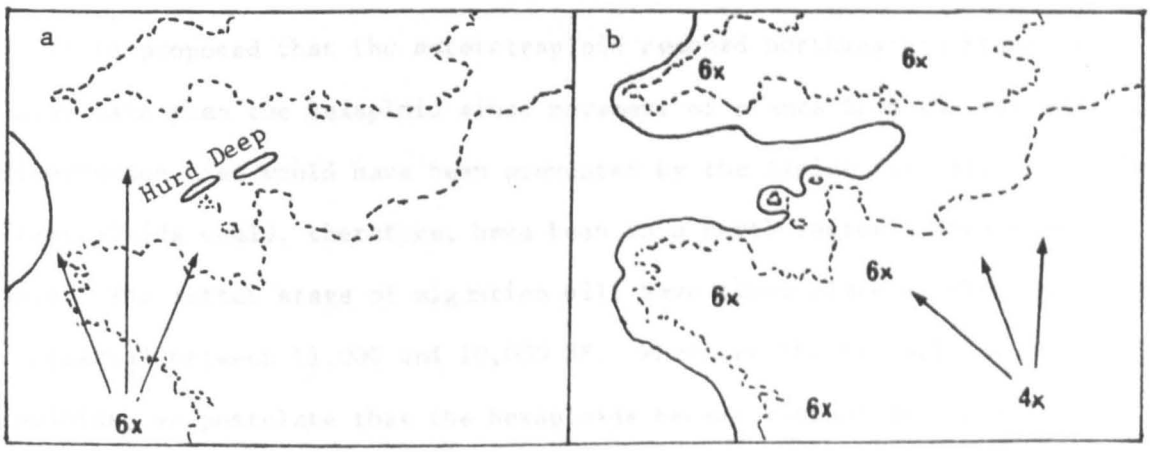
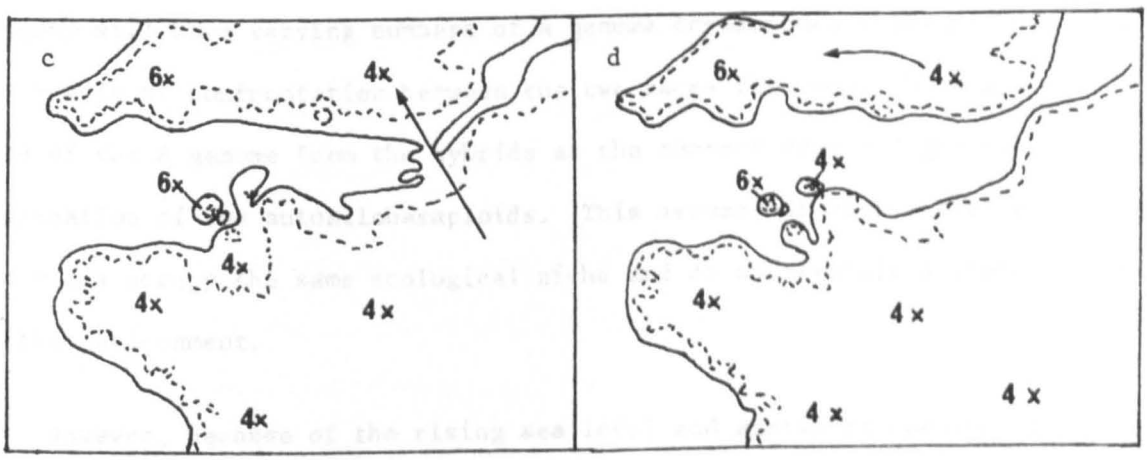


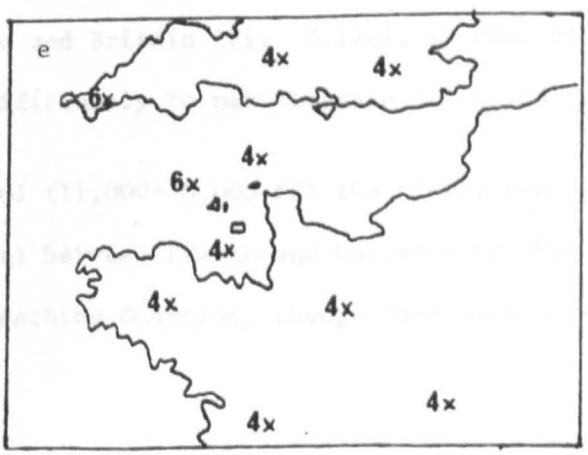
Figure 7.17 A proposed scheme for the colonisation of England and N.W. France by autotetraploid (4x) and autoallohexaploid (6x) races of Scilla autumnalis since the last glaciation. Sea level depths are in parentheses.




15,000 BP (-120 m) 11,000 BP (-55 m)




10,000 BP (-35 m) 8,000 BP (-13 m)




6-7,000 BP (-9 m)

This narrow channel may not have impeded movement of hexaploid plants between Jersey and Guernsey/Sark (Fig. 7.17c). Guernsey and Sark were also separated from each other at about this time.

It is proposed that the autotetraploid reached northwestern France at a later date than the hexaploid since movement of plants from the eastern Mediterranean area would have been prevented by the Alpine ice cap. Migration of tetraploids would, therefore, have been in a north westerly direction into France. The latter stage of migration will have taken place in the Flandrian Interglacial between 11,000 and 10,000 BP. Wherever the tetraploids met the hexaploids, we postulate that the hexaploids became extinct as a result of hybridisation. This would result because of the elimination of A genome chromosomes at meiosis in the $F_1$ hybrid (Fig. 7.13). Subsequent backcross progeny will have varying numbers of A genome chromosomes (between 0 and 7). The result of confrontation between the two races then would be a gradual loss of the A genome from the hybrids at the contact zone and gradual elimination of the autoallohexaploids. This assumes of course that the two races occupy the same ecological niche and do not exploit different facets of the environment.

However, because of the rising sea level and eastwards opening of the English Channel, the tetraploids would have been able to migrate only to south eastern England (Fig. 7.17c). By about 10,000 BP only a small land bridge existed between France and Britain (Fig. 7.17c). By 8000 BP the English Channel had filled sufficiently to make Britain an island (Fig. 7.17d).

During this period (11,000-10,000 BP) the rising sea level caused a widening of the channel between France and Guernsey so that tetraploids were prevented from reaching Guernsey, though they were able to colonise Alderney and Jersey.

The sea depth between Alderney and the Cotentin Peninsula in France is about 35 m. At the beginning of the Flandrian marine transgression (10,000 BP) the depth of the sea was -35 m (Fig. 7.15) this level being exceeded at about 8,000 BP. We can postulate then that the immigration of the tetraploid into Alderney and Jersey took place between 10,000 and 8,000 BP (when Alderney was separated from the mainland) (Fig. 7.17).

At some time between 6 and 7000 BP the sea level rose sufficiently to flood the land bridge between Jersey and Normandy, the present day depth of this channel being only 13 m. Movement of plants from mainland France to Jersey was thus impeded (Fig. 7.17). The sea level rose still further and reached a maximum height of +4 m at about 5000 BP after which time it fell below the present level, and subsequently rose again to it (Fig. 7.15). All movement of plants between France, the Channel Islands and Britain after 8000 BP has thus been prevented. Migration of tetraploid plants in Britain from eastern England westwards, however, may still have continued.

The presence of both autoallohexaploid and tetraploids in southern England might appear to contradict the theory of the elimination of hexaploid populations by hybridisation with tetraploids. This is not the case and the hexaploid populations in Cornwall are, at the present time, ecologically isolated from the tetraploid populations to the east, by a lack of suitable sites for Scilla colonisation. One has to make certain assumptions to explain this distribution. The first is that the tetraploids colonised Britain starting from a point well to the east of the present hexaploid populations in Cornwall which appears likely from the sea level data, and then migrated westwards, hybridising with and eliminating hexaploid populations. The second is that the areas of unsuitable habitat between hexaploid and tetraploid populations were present at 10,000 BP. There is no evidence to contradict this latter assumption and the coastal areas of north and south Cornwall have

probably remained relatively undisturbed since then.

Corroborative evidence for this scheme comes from consideration of the distribution of the Inv 3-1 polymorphism in north-western France and Britain. This inversion reaches polymorphic proportions in both tetraploid and auto-allohexaploid populations (Fig. 6.9 , 6.10). Amongst tetraploid populations there is a clear clinal distribution with the highest frequencies in the northernmost populations. From an evolutionary standpoint there are two possible explanations of this phenomenon: i) the inversion arose initially in the tetraploid and predates the evolution of the hexaploid, ii) the inversion arose in the autoallohexaploid and has been transferred to the hexaploid by hybrisisation. The absence of Inv 3-1 from Corfu tetraploids and allotetraploids and the nature of the cline itself suggests that the latter is the more likely explanation. If this is so, then tetraploid populations where Inv 3-1 is now present mark relict hexaploid populations. Thus, we infer that at some time in the past the hexaploid was present over the whole of the area of north western France and southern England now occupied by tetraploids.

II. The relationship between A and B genomes

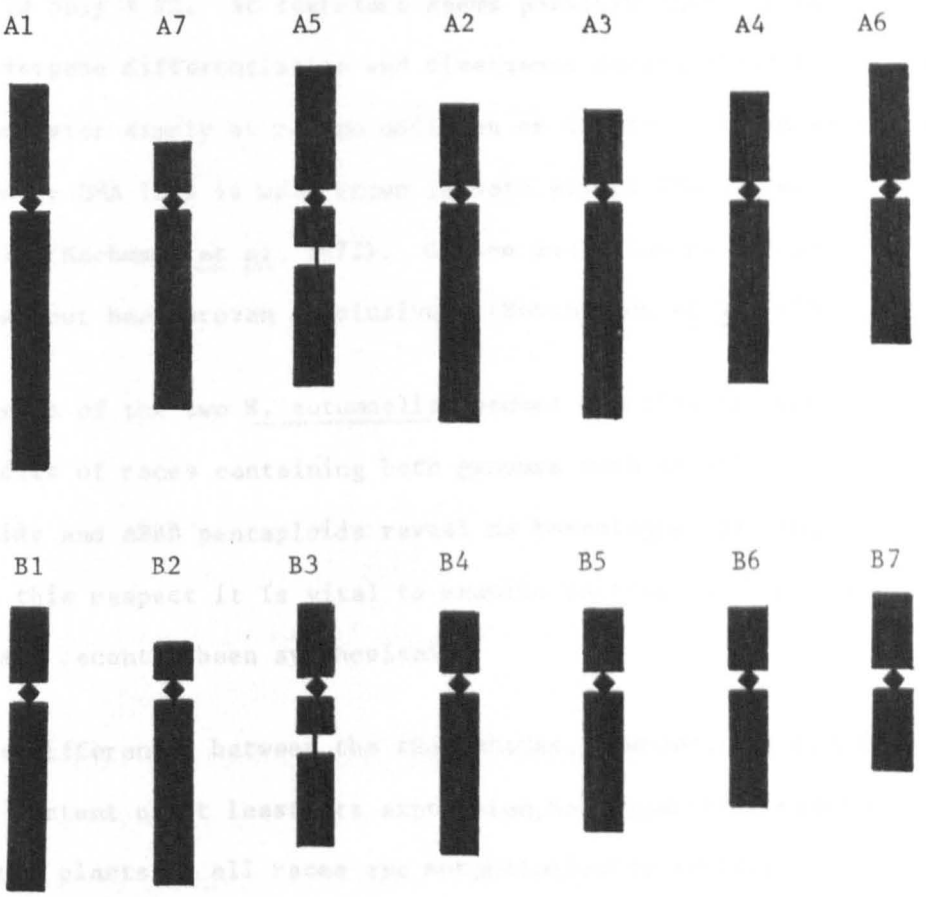
The A genome is 43% longer with wider chromosomes than the B genome. Although the karyotypes have previously been arranged on the basis of chromosome length (Fig. 3.8) they can also be considered in terms of their morphological similarity (Fig.7.18). Remarkably the respective genomes are very similar and each genome consists of

i) a nucleolar-organiser chromosome with the NOR interstitial and close to the centromere;

ii) a highly acrocentric chromosome with an arm ratio of about 1.5;

iii) a metacentric chromosome;

Figure 7.18 A comparison of the karyotypes of the A and B genomes of S. autumnalis based on chromosome morphology




| Difference | A1-B1 | A7-B2 | A5-B3 | A2-B4 | A3-B5 | A4-B6 | A6-B7 |
|---|---|---|---|---|---|---|---|
| in length (μm) | 3.74 | 0.74 | 2.71 | 2.86 | 3.15 | 3.34 | 3.79 |
| (%) | 30.4 | 9.22 | 29.4 | 28.4 | 32.5 | 36.7 | 43.5 |

iv) a large acrocentric with an arm ratio of about 1:2;

v) three smaller acrocentrics with arm ratios of about 1:2.

Considering each chromosome separately, the differences in length between the proposed A and B counterparts vary between 0.74 and 3.79 μm and on average the A genome chromosomes are about 30% longer than the corresponding B genome chromosome (Fig. 7.18). A single exception is chromosome 2 where the difference is only 9.2%. It therefore seems possible that the two genomes have undergone differentiation and divergence during their evolution from a common ancestor simply by random addition or deletion of DNA segments. Genome evolution by DNA loss is well known in both plants (Hutchinson et al, 1980) and animals (Bachmann et al, 1972). Genome evolution by DNA gain (duplication) has not been proven conclusively (Hutchinson et al, 1980).

The divergence of the two S. autumnalis genomes has clearly been considerable. Studies of races containing both genomes such as allotetraploids, autoallohexaploids and ABBB pentaploids reveal no homeologous pairing whatsoever. In this respect it is vital to examine pairing in AB diploid hybrids which have recently been synthesised.

Despite the differences between the two genomes, however, the overall structural gene content or at least its expression, has apparently remained very similar since plants of all races are morphologically extremely similar, even the AA and BB diploids.

In the evolution of the complex there has, therefore, been an early primary divergence of the two genomic types followed by a more recent convergence through the polyploid complex. In the case of the primary divergence it is impossible to tell whether the two genomes have evolved from a common ancestor or whether one gave rise to the other.

Polyploid complexes have been well documented in plants (Stebbins, 1971) but the best parallels to S. autumnalis are to be found in the wheat and Scilla scilloides complexes. The various species of Triticum (including Aegilops) from a polyploid series with x = 7 consisting of diploids, tetraploids and hexaploids. The wild diploids, which are presumably monophyletic in origin, have diverged considerably from each other, each diploid containing a distinct genome showing little homoeologous pairing in hybrids (Kihara, 1954). The polyploid wheats are allopolyploids and each species can be identified as a product of hybridisation and chromosome doubling. The diploid-like behaviour of the polyploid wheats is due to suppression of homoeologous pairing by a specific locus Ph, located on the long arm of chromosome 5 of the B genome (Riley, 1965). It is possible that a similar system operates in S. autumnalis preventing homoeologous pairing between genically-similar chromosomes.

The situation in Scilla scilloides is more akin to that in Scilla autumnalis. Two cytogenetically well differentiated genomes occur which have come together to form a series of auto- and allo-polyploids, collectively referred to as S. scilloides (Noda, 1967). In contrast to those in S. autumnalis, however, the two S. scilloides genomes are differentiated by an aneuploid difference (x = 8 and x = 9) but are to some degree homologous. In S. scilloides, populations generally contain a mixture of many cytological types, in contrast to S. autumnalis where populations are always made up of a single cytological race.

Despite natural hybridisation between Scilla bifolia and Chionodoxa luciliae hybridisation between other members of the genus Scilla is rare and strong interspecific barriers are present between some species. For example, S.monophyllos (2n = 20) and S. ramburei (2n = 20) have very

similar karyotypes, flower concurrently and often grow in very close proximity yet hybridisation between them does not occur (Parker and Ainsworth).

Scilla autumnalis shares with many other polyploid complexes of the north temperate region a relationship between increasing ploidy and increasing latitude. This was initially attributed by Müntzing (1936) to the greater hardiness of polyploids. This theory has largely been discounted (Nielsen, 1947; Gustafsson, 1948) and it is a more reasonable assumption that polyploids have evolved as a result of novel hybridisations during northward plant movements after the last glaciation. Darlington (1963) has argued cogently for such a relationship between movement and chromosome evolution.

Prior to this study no distinction has been drawn between the A and B genome although it is probable that counts of 2n = 28 recorded by Battaglia (1964) in Portuguese material were of the allotetraploid race. We must, therefore, view with considerable circumspection the spatial distribution of this polyploid complex revealed by previous work. It is possible, for example, that an autotetraploid race AAAA may occur in Europe or Africa. Clearly, further studies of this complex are required across the whole range of the distribution. A particularly fruitful field of study would be the Iberian peninsula in which four distinct races - AA, BB, AABB and BBBB - are at present known.

# CHAPTER EIGHT

## GENERAL DISCUSSION

Prior to the inception of this study some cytological work had been carried out on Scilla autumnalis but it was in the main rather a haphazard sampling of one or two bulbs from isolated populations to determine the level of ploidy. The existence of a polyploid series was noted by Battaglia who from examination of single plants from populations in the Mediterranean region recognised diploids, autotetraploids and autohexaploids (Battaglia, 1952, 1957, 1964). An autoallohexaploid plant recorded as from south Devon (certainly incorrectly) was karyotyped and illustrated but the significance of the A genome was missed. Chromosomal variation within a cytological race, whilst its presence was noted by Battaglia, was dismissed as being of "no importance" (Battaglia, 1964). However, this study of Scilla autumnalis has shown that it is a quite remarkable organism from a cytological standpoint. In contrast to the simple autopolyploid series envisaged by Battaglia, the species is a polyploid complex comprising six cytologically-distinct races which exhibit quite unprecedented levels of both structural and numerical variation.

Numerical variation, encountered at three levels of ploidy (diploid, autotetraploid and autoallohexaploid) comprised aneusomaty, aneuploidy, polysomaty and polyploidy. The degree of numerical variation, both intra-individual and inter-individual, increased with ploidy level suggesting both that meiotic and mitotic errors are more frequent in plants with larger numbers of chromosomes and that higher polyploids are better able to tolerate the genetic imbalance due to chromosomal buffering. Aneuploidy affected all chromosome groups equally although

chromosome B2 was involved in aneuploidy most frequently. Interestingly this chromosome was also most subject to deletion. In autoallohexaploid plants there is no evidence to suggest that A or B genome chromosomes are more prone to involvement in aneuploidy. In addition to numerical variation in the standard chromosomes of Scilla autumnalis two tetraploid populations contained euchromatic B-chromosomes. B-chromosomes appear rather frequent in Scilla autumnalis and have also been recorded in AA (Parker, pers. com.) and BB diploids.

Structural variation in Scilla autumnalis was of three types: spontaneous between-cell, whole plant unique and whole plant polymorphic. The level of spontaneous variation correlates well with the levels found in other species, and, as for numerical variants, there was an increase in the level of variation with ploidy level. The distribution of spontaneous variation was equally spread amongst the chromosome groups. By contrast, chromosome B3, the nucleolar-organiser chromosome, was particularly affected in whole plant variation. Nucleolar-organiser chromosomes are well known in many organisms to be susceptible to structural rearrangement. Chromosome 4, on the other hand, was particularly invariant perhaps suggesting that some rearrangements affecting this chromosome are lethal.

Of the structural changes affecting whole plants, deletions, inversions and duplications were common but only the latter two were involved in polymorphic systems. Interchanges were unexpectedly rare. The incidence of non-polymorphic (unique) structural variation increases with ploidy level, although the trend is reversed if the actual number of chromosomes is taken into account.

Polymorphic variation was greatest in the diploids and least in the hexaploids. However, when considering the detection of structural

variants it must be borne in mind that with increased numbers of chromosomes per cell it becomes more difficult to detect structural change and in particular those of small size. In addition, due to the mitotic nature of the analysis, estimates of the frequency of structural variants are conservative since symmetrical exchanges and most paracentric inversions cannot be detected at mitosis.

The extent of polymorphic variation in Scilla autumnalis is quite remarkable with several polymorphisms being extremely widespread. The frequency of one inversion polymorphism (Inv 3-1) was clinal with respect to latitude, increasing towards the northern extreme of the distribution. This is particularly interesting in view of the fact that the overall incidence of polymorphism showed a significant negative relationship with latitude, suggesting a central-peripheral effect as in Drosophila. Inversion 3-1 presumably confers a considerable selective advantage to individuals carrying it in southern England.

About 43% of 1490 plants karyotyped (diploids, autotetraploids and autoallohexaploids) were structurally variant. When numerical variation is included, the total of non-standard plants rises to 50%. Is this a typical situation in the genus Scilla or indeed in plants in general? Little population data on Scilla species exists with the exception of Scilla peruviana studied by Battaglia and co-workers (Battaglia, 1948, 1949, 1951; Battaglia, Cesca & Maggini, 1969). Scilla peruviana (2n = 2x = 16) appears chromosomally variable and "numerous chromosomal mutations" were recorded, particularly in the N.O. chromosome. Without population scores, however, the extent of variation cannot be assessed. It is also impossible to tell whether the variation of the N.O. region is simply satellite variation, as is commonly observed, or true structural rearrangement. Analysis of a

few plants of S. bifolia by the author has indicated that chromosomal variation due to heterochromatic segments may be frequent as it is in Scilla sibirica (Vosa, 1973). The polyploid complex of Scilla scilloides shows little structural variation (Noda, 1967). , analysis of Scilla verna populations in Britain and France has revealed very little variation (Taylor, pers. comm.). Thus, no other Scilla species shows the level of structural variation exhibited by Scilla autumnalis.

Bulbous species in general and members of the family Liliaceae in particular seem prone to chromosomal variation. This may in part reflect the fact that chromosomes of Liliaceae are particularly amenable to cytological work so that considerable effort has been expended on this family, perhaps second only to the Gramineae. By contrast, however, only three structural variants have been detected in Hypochoeris (Compositae) in 14 years of study in this laboratory (Parker, pers. comm.). Both Allium schoenoprasum (Bougourd, 1977) and Scilla autumnalis have shown chromosomal variation several orders of magnitude greater than this. Perhaps the bulbous habit is in some way associated with persistence of chromosomal variants.

The Scilla autumnalis complex includes six races and two genomes both with x = 7. Despite the fact that the A and B genomes must express similar genetic information (the two diploids, AA and BB appear identical in morphology and ecological preference) no homology is demonstrated between the two genomes, even in allotetraploids. In addition, the A genome is 43% longer than the B genome, yet the chromosomes of both are of very similar morphology. This is markedly different from the situation in the S. scilloides complex where the two genomes of x = 8 and x = 9 are morphologically very different, yet are still segmentally homologous (Araki, 1971).

In both species it seems that there has been an ancient primary difergence of the two genomes giving the diploids AA and BB, followed by a much more recent convergence as a result of intense hybridisation leading to the formation of the polyploid complex. A part of the evolutionary history of the S. autumnalis complex, the present day distribution of the autoallohexaploid, can be correlated with the recent paleo-geographical history of the north-western seaboard of Europe.

The extraordinary amount of chromosomal variation present, and still being generated, in S. autumnalis may be a consequence of the rapid increase in population size which will have occurred due to climatic amelioration in the Quaternary era. Rapidly increasing numbers in populations are associated with levels of high variation (Mather, 1953) as observed by Ford and Ford (1930) in the butterfly Melitaea aurinea. As Darlington (1956) concluded: "Not size but change of size is what matters most". The wealth of chromosomal variation so generated is an effective mechanism for testing new combinations of genes against the new associations of genomes brought about by hybridisation and chromosome doubling. In view of the many and widespread polymorphisms discovered it must be concluded that some of these gene combinations are particularly effective in certain environments.

The similarities and differences between the two genomes in S. autumnalis are in themselves fascinating, particularly their 43% difference in length. The difference between the two genomes is likely to be still greater in terms of volume taking into account the slightly greater width of the A genome chromosomes. The largest difference reported in chromosome volume between genomes involved in hybridisation is in Lolium perenne x temulentum hybrids where the di rence in chromosome length is 32% and volume 40%. The DNA difference was 37% (Rees and Jones, 1967). In Lolium it was

concluded that the change was at least in part due to duplication of chromosome segments, the change being widespread throughout the complement (Rees and Jones, 1967). In S. autumnalis also, the changes are distributed throughout all the chromosome groups. Deletion or duplication of such a large amount of DNA (all of which is euchromatic in the present day genomes), if it were restricted to a few chromosomes within the complement, would be unlikely to be tolerated since severe genetic imbalance would result, at least in diploids.

There is evidence that the quantitative DNA changes which take place during evolution implicate a particular and specific DNA fraction, termed supplementary DNA (Hutchinson et al, 1980). The composition of this extra supplementary DNA has been found to be very similar within families and even genera. In most species analysed in this respect, the greatest change in DNA has been in the heterochromatin which is in direct contrast to the situation in Scilla autumnalis (e.g. Rees and Narayan, 1977; Burns and Gerstel, 1969).

Organisms show considerable interspecific variation in their DNA contents and although there is a general trend for more complex organisms to have more DNA this is by no means absolute. It is in the higher plants and animals that the C-value paradox presents itself most forcibly since species have widely disparate DNA contents. For example Fritillaria davisii has over 30 times more DNA per 2C nucleus than Homo sapiens (Rees and Jones, 1977) and even closely related species can differ widely, Vicia faba having seven times more DNA than V. sativa. Such variation in DNA content must be linked with the actual organisation of DNA in relation to the relative proportions of unique and repetitive DNA. The general pattern of organisation of eukaryote DNA is that short repetitive elements of about 300 nucleotides are interspersed throughout a large fraction of

single-copy DNA at intervals of 800 to several thousand nucleotide pairs (Davidson et al. 1975; Walbot and Goldberg, 1978). In plants a larger fraction of DNA sequences are repetitive (Flavell et al, 1974; Walbot and Goldberg, 1978). Flavell et al (1979) have shown that in Tritium and Aegilops the repeated sequence complement of the different genomes are very similar. Similar analyses on the A and B genomes of Scilla autumnalis to reveal the nature of the DNA change in the evolution of the genomes would be invaluable. In the light of the large difference in DNA content between the two genomes, which is not heterochromatic in nature, such study might provide an insight into the C-value paradox.

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# APPENDIX I

## Cytological Methods

### a) Mitosis

Analysis of karyotypes was made on root-tips. Excised root tips were pre-treated in 0.05% colchicine solution for 3½ hours at room temperature and fixed in glacial acetic acid:absolute ethanol (1:3). Fixed root-tips were hydrolysed for 10 minutes in N HCl at 60°C before staining with Feulgen (Darlington and La Cour, 1976). The cells were dissociated by tapping with a brass rod and counterstained with lacto-propionic orcein (Dyer, 1963).

Five cells from two different roots were counted where possible for each plant.

### b) Meiosis

Preparations of PMCs were made with both fresh and fixed material. In the latter case, the anthers were dissected out of buds and fixed in Newcomer's non-polar fixing fluid (Newcomer, 1952). The anthers were stored at 4° for 24 hours after which time the fixing fluid was changed. Thereafter, the anthers were stored at -10°C. Single anthers were gently dissociated in a drop of lacto-propionic orcein before carefully lowering a cover slip onto the slide. Slides were stored overnight at 4°C to allow the fragile PMC's to harden before gently squashing.

Preparations were retained where necessary as temporary mounts ringed with rubber solution and stored at -10°C.

# APPENDIX II

## Abbreviations

| | |
|---|---|
| I, II, III etc. | univalent, bivalent, trivalent etc. |
| M-I/M-II | metaphase-I/II |
| A-I/A-II | anaphase-I/II |
| PMC | pollen mother cell |
| C-metaphase | colchicine-metaphase |
| het. | heterozygote |
| del. | deletion |
| dup. | duplication |
| int. | interchange |
| inv. | inversion |
| N.O./NOR | nucleolar-organiser/nucleolar-organiser region |
| rDNA | ribosomal DNA |

## ACKNOWLEDGEMENTS

The research on which this thesis is based was completed during the tenure of an S.R.C. award at the Department of Plant Biology and Microbiology, Queen Mary College. The author gratefully acknowledges the financial support of the Science Research Council and the facilities made available by Professor E.A. Bevan.

I should like to express my sincere gratitude to Dr. John Parker for his unfailing encouragement, guidance and enthusiasm during the research towards, and preparation of this thesis.

I am particularly indebted to Mrs. Jean Smith for so painstakingly typing this thesis and to Mrs. Lesley Ainsworth for her invaluable assistance in the preparation of it.

I also wish to thank Dr. R.S. Callow for his most helpful statistical advice.